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(56) References cited:  
US-A- 4 666 828

- SOMAT. CELL MOL. GENET., vol. 17, no. 5, 1991  
pages 481-488, LIN ET AL. 'New DNA markers in  
the Huntington's disease gene candidate region'
- NATURE GENET., vol. 1, May 1992 pages 99-103,  
MAC DONALD ET AL. 'The Huntington's disease  
candidate region exhibits many different  
haplotypes'
- CELL, vol. 72, 26 March 1993 pages 971-983, THE  
HUNTINGTON'S DISEASE COLLABORATIVE  
RESEARCH GROUP 'A novel gene containing a  
trinucleotide repeat that is expanded and  
unstable on Huntington's disease  
chromosomes'
- CR ACAD. SCI. III, vol. 316, no. 11, November  
1993 pages 1374-1380, DOD ET AL.  
'Huntington's disease in French families : CAG  
repeat expansion and linkage disequilibrium  
analysis'
- MOL. CELL. PROBES, vol. 7, no. 3, June 1993  
pages 235-239, WARNER ET AL. 'A new  
polymerase chain reaction (PCR) assay for the  
trinucleotide repeat that is unstable and  
expanded on Huntington's disease  
chromosomes'
- MOL. CELL. BIOL., vol. 10, no. 11, November  
1990 pages 5616-5625, LAURENT ET AL. 'The  
SNF5 protein of *Saccharomyces cerevisiae* is a  
glutamine- and proline-rich transcriptional  
activator that affects expression of a broad  
spectrum of genes'

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**Description****Field of the Invention**

5 [0001] The invention is in the field of the detection and treatment of genetic diseases. Specifically, the invention is directed to the *huntingtin* gene (also called the IT15 gene), huntingtin protein encoded by such gene, and the use of this gene and protein in assays (1) for the detection of a predisposition to develop Huntington's disease, (2) for the diagnosis of Huntington's disease (3) for the treatment of Huntington's disease, and (4) for monitoring the course of treatment of such treatment.

10

**Background of the Invention**

15 [0002] Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by motor disturbance, cognitive loss and psychiatric manifestations (Martin and Gusella, *N. Engl. J. Med.* 315:1267-1276 (1986)). It is inherited in an autosomal dominant fashion, and affects about 1/10,000 individuals in most populations of European origin (Harp-  
er, P.S. *et al.*, in *Huntington's disease*, W.B. Saunders, Philadelphia, 1991). The hallmark of HD is a distinctive choreic movement disorder that typically has a subtle, insidious onset in the fourth to fifth decade of life and gradually worsens over a course of 10 to 20 years until death. Occasionally, HD is expressed in juveniles typically manifesting with more severe symptoms including rigidity and a more rapid course. Juvenile onset of HD is associated with a preponderance  
20 of paternal transmission of the disease allele. The neuropathology of HD also displays a distinctive pattern, with selective loss of neurons that is most severe in the caudate and putamen regions of the brain. The biochemical basis for neuronal death in HD has not yet been explained, and there is consequently no treatment effective in delaying or preventing the onset and progression of this devastating disorder.

25 [0003] The genetic defect causing HD was assigned to chromosome 4 in 1983 in one of the first successes of linkage analysis using polymorphic DNA markers in man (Gusella *et al.*, *Nature* 306:234-238 (1983)). Since that time, we have pursued a location cloning approach to isolating and characterizing the HD gene based on progressively refining its localization (Gusella, *FASEB J.* 3:2036-2041 (1989); Gusella, *Adv. Hum. Genet.* 20:125-151 (1991)). Among other work, this has involved the generation of new genetic markers in the region by a number of techniques (Pohl *et al.*, *Nucleic Acids Res.* 16:9185-9198 (1988); Whaley *et al.*, *Somat. Cell. Mol. Genet.* 17:83-91 (1991); MacDonald *et al.*, *J. Clin. Inv.* 84:1013-1016 (1989)), the establishment of genetic (MacDonald *et al.*, *Neuron* 3:183-190 (1989); Allitto *et al.*, *Genomics* 9:104-112 (1991)) and physical maps of the implicated regions (Bucan *et al.*, *Genomics* 6:1-15 (1990); Bates *et al.*, *Nature Genet.* 1:180-187 (1992); Doucette-Stamm *et al.*, *Somat. Cell Mol. Genet.* 17:471-480 (1991); Altherr *et al.*, *Genomics* 13:1040-1046 (1992)), the cloning of the 4p telomere of an HD chromosome in a YAC clone (Bates *et al.*, *Am. J. Hum. Genet.* 46:762-775 (1990); Youngman *et al.*, *Genomics* 14:350-356 (1992)), the establishment  
30 of YAC [yeast artificial chromosome] (Bates *et al.*, *Nature Genet.* 1:180-187 (1992)) and cosmid (Baxendale *et al.*, in preparation) contigs (a series of overlapping clones which together form a whole sequence) of the candidate region, as well as the analysis and characterization of a number of candidate genes from the region (Thompson *et al.*, *Genomics* 11:1133-1142 (1991); Taylor *et al.*, *Nature Genet.* 2:223-227 (1992); Ambrose *et al.*, *Hum. Mol. Genet.* 1:697-703 (1992)). Analysis of recombination events in HD kindreds has identified a candidate region of 2.2 Mb, between D4S10  
35 and D4S98 in 4p16.3, as the most likely position of the HD gene (MacDonald *et al.*, *Neuron* 3:183-190 (1989); Bates *et al.*, *Am. J. Hum. Genet.* 49:7-16 (1991); Snell *et al.*, *Am. J. Hum. Genet.* 51:357-362 (1992)). Investigations of linkage disequilibrium between HD and DNA markers in 4p16.3 (Snell *et al.*, *J. Med. Genet.* 26:673-675 (1989); Theilman *et al.*, *J. Med. Genet.* 26:676-681 (1989)) have suggested that multiple mutations have occurred to cause the disorder (MacDonald *et al.*, *Am. J. Hum. Genet.* 49:723-734 (1991)). However, haplotype analysis using multi-allele markers  
40 has indicated that at least 1/3 of HD chromosomes are ancestrally related (MacDonald *et al.*, *Nature Genet.* 1: 99-103 (1992)). The haplotype shared by these HD chromosomes points to a 500 kb segment between D4S180 and D4S182 as the most likely site of the genetic defect.

45 [0004] Targeting this 500 kb region for saturation with gene transcripts, exon amplification has been used as a rapid method for obtaining candidate coding sequences (Buckler *et al.*, *Proc. Natl. Acad. Sci. USA* 88:4005-4009 (1991)). This strategy has previously identified three genes: the a-adducin gene (ADDA) (Taylor *et al.*, *Nature Genet.* 2:223-227 (1992)); a putative novel transporter gene (IT10C3) in the distal portion of this segment; and a novel G protein-coupled receptor kinase gene (IT11) in the central portion (Ambrose *et al.*, *Hum. Mol. Genet.* 1:697-703 (1992)). However, no defects implicating any of these genes as the HD locus have been found.

**55 Summary of the Invention**

[0005] A large gene, termed herein "huntingtin" or "IT15," has been identified that spans about 210 kb and encodes a previously undescribed protein of about 348 kDa. The huntingtin reading frame contains a polymorphic (CAG)<sub>n</sub> tri-

nucleotide repeat with at least 17 alleles in the normal population, varying from 11 to about 34 CAG copies. On HD chromosomes, the length of the trinucleotide repeat is substantially increased, for example about 37 to at least 73 copies, and shows an apparent correlation with age of onset, the longest segments are detected in juvenile HD cases. The instability in length of the repeat is reminiscent of similar trinucleotide repeats in the fragile X syndrome and in myotonic dystrophy (Suthers *et al.*, *J. Med. Genet.* 29:761-765 (1992)). The presence of an unstable, expandable trinucleotide repeat on HD chromosomes in the region of strongest linkage disequilibrium with the disorder suggests that this alteration underlies the dominant phenotype of HD, and that huntingtin encodes the HD gene.

5 [0006] The invention is directed to the protein huntingtin, DNA and RNA encoding this protein, and uses thereof.

10 [0007] According to a first aspect of the present invention, there is provided an isolated, purified or recombinant huntingtin polypeptide comprising the amino acid sequence shown in SEQ ID NO:6.

[0008] According to a second aspect of the present invention, there is provided an isolated, purified or recombinant nucleic acid molecule comprising a huntingtin nucleic acid molecule encoding a huntingtin polypeptide in accordance with the first aspect of the invention, or its complementary strand.

15 [0009] Preferably, the nucleic acid molecule comprises the nucleic acid shown in SEQ ID NO:5, and may comprise a transcriptional control region operably linked to the huntingtin nucleic acid molecule.

[0010] The invention also provides, in a third aspect, a vector comprising a nucleic acid molecule of the second aspect of the invention. In this vector, the nucleic acid molecule may be operably linked to transcriptional and/or translational expression signals.

20 [0011] In a fourth aspect, the invention provides a host cell transformed or transfected with a vector according to the third aspect of the invention.

[0012] In fifth and sixth aspects, the invention also provides an antibody specific for huntingtin polypeptide of the first aspect, and a hybridoma which produces such an antibody.

25 [0013] According to a seventh aspect, the invention provides a method of detecting the presence of, or predisposition to develop, Huntington's disease in a subject, the method comprising

(a) evaluating the characteristics of huntingtin nucleic acid in a sample from the subject, wherein the evaluation comprises detecting the huntingtin (CAG)<sub>n</sub> region shown in SEQ ID NO:5 in the sample; and

30 (b) comparing the characteristics found in (a) with a similar analysis from an individual with no family history of Huntington's disease, where the nucleic acid has from 11 to 34 (CAG) repeats, the presence of, or predisposition to develop, Huntington's disease being indicated if those characteristics in the huntingtin (CAG)<sub>n</sub> region differ. The characteristics of huntingtin nucleic acid may be evaluated by Southern blot, northern blot, or polymerase chain reaction analysis.

35 [0014] In an eighth aspect, the invention provides the use of:

(a) a nucleic acid molecule of the second aspect or a vector of the third aspect;  
 (b) a polypeptide of the first aspect; and/or  
 (c) a host cell of the fourth aspect

40 in the preparation of a medicament.

[0015] The medicament may be for treating, delaying or preventing a neurodegenerative disorder, preferably Huntington's disease, and may be for gene therapy. Preferably, the nucleic acid has from 11 to 34 (CAG) repeats and/or the polypeptide has from 11 to 34 Gln repeats, said repeats being consecutive.

45 [0016] In a further aspect, the invention provides a diagnostic and/or immunoassay kit comprising at least one container and;

(a) a nucleic acid molecule of the second aspect, optionally labelled; or  
 (b) an antibody of the fifth aspect, optionally labelled.

50 [0017] In a still further aspect, the invention provides a pharmaceutical composition comprising:

(a) a nucleic acid molecule of the second aspect or a vector of the third aspect;  
 (b) a polypeptide of the first aspect; and/or  
 (c) a host cell of the fourth aspect

55 in admixture with pharmaceutically acceptable carrier.

[0018] In a yet further aspect, the invention provides process for the preparation of a polypeptide of the first aspect of the invention, the process comprising culturing a host cell according to of the fourth aspect under conditions whereby

the polypeptide is expressed, and purifying or isolating the polypeptide.

[0019] A functional *huntingtin* gene with a (CAG)<sub>n</sub> repeat of the normal range of 11-34 copies may be used in the manufacture of a medicament for gene therapy of a symptomatic or presymptomatic patient, the medicament being for administration to the desired cells of such patient in need of such treatment, in a manner that permits the expression of the huntingtin protein provided by such gene, for a time and in a quantity sufficient to provide the huntingtin function to the cells of such patient. Alternatively, a functional *huntingtin* antisense gene may be used in the manufacture of such a medicament which is for administration to the desired cells of such patient in need of such treatment, in a manner that permits the expression of huntingtin antisense RNA provided by such gene, for a time and in a quantity sufficient to inhibit huntingtin mRNA expression in the cells of such patient, or a functional *huntingtin* gene may be used in the manufacture of such a medicament which is for administration to the cells of such patient in need of such gene; the functional huntingtin gene may contain a (CAG)<sub>n</sub> repeat size between 11-34 copies.

[0020] A method for diagnosing Huntington's disease or a predisposition to develop Huntington's disease in a patient, may comprise determining the number of (CAG)<sub>n</sub> repeats present in the huntingtin gene in such patient and especially in the affected tissue of such patient.

[0021] Huntington's disease may be treated in a patient, decreasing the number of huntingtin (CAG)<sub>n</sub> repeats in the huntingtin gene in the desired cells of such patient.

#### ***Brief Description of the Drawings***

[0022] FIGURE 1. Long-range restriction map of the *HD* candidate region. A partial long range restriction map of 4p16.3 is shown (adapted from Lin *et al.*, *Somat. Cell Mol. Genet.* 17:481-488 (1991)). The *HD* candidate region determined by recombination events is depicted as a hatched line between *D4S10* and *D4S98*. The portion of the *HD* candidate region implicated as the site of the defect by linkage disequilibrium haplotype analysis (MacDonald *et al.*, *Nature Genet.* 1:99-103 (1992)) is shown as a filled box. Below the map schematic, the region from *D4S180* to *D4S182* is expanded to show the cosmid contig (averaging 40 kb/cosmid). The genomic coverage and where known the transcriptional orientation (arrow 5' to 3') of the huntingtin (IT15), IT11, IT10C3 and ADDA genes is also shown. Locus names above the map denote selected polymorphic markers that have been used in HD families. The positions of *D4S127* and *D4S95* which form the core of haplotype in the region of maximum disequilibrium are also shown in the cosmid contig. Restriction sites are given for Not I (N), Mlu I (M) and Nru I (R). Sites displaying complete digestion are shown in boldface while sites subject to frequent incomplete digestion are shown as lighter symbols. Brackets around the "N" symbols indicate the presence of additional clustered Not I sites.

[0023] FIGURE 2. Northern blot analysis of the huntingtin (IT15) transcript. Results of the hybridization of IT15A to a Northern blot of RNA from normal (lane 1) and HD homozygous (lane 2 and 3) lymphoblasts are shown. A single RNA of about 11 kb was detected in all three samples, with slight apparent variations being due to unequal RNA concentrations. The HD homozygotes are independent, deriving from the large an American family (lane 2) and the large Venezuelan family (lane 3), respectively. The Venezuelan HD chromosome has a 4p16.3 haplotype of "5 2 2" defined by a (GT)<sub>n</sub> polymorphism at *D4S127* and VNTR and TaqI RFLPs at *D4S95*. The American homozygote carries the most common 4p16.3 haplotype found on HD chromosomes: "2 11 1" (MacDonald *et al.*, *Nature Genet.* 1:99-103 (1992)).

[0024] FIGURE 3. Schematic of cDNA clones defining the IT15 transcript. Five cDNAs are represented under a schematic of the composite IT15 sequence. The thin line corresponds to untranslated regions. The thick line corresponds to coding sequence, assuming initiation of translation at the first Met codon in the open reading frame. Stars mark the positions of the following exon clones 5' to 3': DL83D3-8, DL83D3-1, DL228B6-3, DL228B6-5, DL228B6-13, DL69F7-3, DL178H4-6, DL118F5-U and DL134B9-U4.

[0025] The composite sequence was derived as follows. From 22 bases 3' to the putative initiator Met ATG, the sequence was compiled from the cDNA clones and exons shown. There are 9 bases of sequence intervening between the 3' end of IT16B and the 5' end of IT15B. These were by PCR amplification of first strand cDNA and sequencing of the PCR product. At the 5' end of the composite sequence, the cDNA clone IT16C terminates 27 bases upstream of the (CAG)<sub>n</sub>. However, when IT16C was identified, we had already generated genomic sequence surrounding the (CAG)<sub>n</sub> in an attempt to generate new polymorphisms. This sequence matched the IT16C sequence, and extended it 337 bases upstream, including the apparent Met initiation codon.

[0026] FIGURE 4. Composite sequence of huntingtin (IT15)(SEQ ID NO:5 and SEQ ID NO:6). The composite DNA sequence of huntingtin (IT15) is shown (SEQ ID NO:5). The predicted protein product (SEQ ID NO:6) is shown below the DNA sequence, based on the assumption that translation begins at the first in-frame methionine of the long open reading frame.

[0027] FIGURE 5. DNA sequence analysis of the (CAG)<sub>n</sub> repeat. DNA sequence shown in panels 1, 2 and 3, demonstrates the variation in the (CAG)<sub>n</sub> repeat detected in normal cosmid L191F1 (1), cDNA IT16C (2), and *HD* cosmid GUS72-2130. Panels 1 and 3 were generated by direct sequencing of cosmid subclones using the following primer

(SEQ ID NO:1):

5' GGC GGG AGA CCG CCA TGG CG 3'.

5

Panel 2 was generated using the pBSKII T7 primer (SEQ ID NO:2):

10 5' AAT ACG ACT CAC TAT AG 3'.

[0028] FIGURE 6. PCR analysis of the (CAG)<sub>n</sub> repeat in a Venezuelan HD sibship with some offspring displaying juvenile onset. Results of PCR analysis of a sibship in the Venezuela HD pedigree are shown. Affected individuals are represented by shaded symbols. Progeny are shown as triangles for confidentiality. AN1, AN2 and AN3 mark the positions of the allelic products from normal chromosomes. AE marks the range of PCR products from the *HD* chromosome. The intensity of background constant bands, which represent a useful reference for comparison of the above PCR products, varies with slight differences in PCR conditions. The PCR products from cosmids L191F1 and GUS72-2130 are loaded in lanes 12 and 13 and have 18 and 48 CAG repeats, respectively.

[0029] FIGURE 7. PCR analysis of the (CAG)<sub>n</sub> repeat in a Venezuelan HD sibship with offspring homozygous for the same *HD* haplotype. Results of PCR analysis of a sibship from the Venezuela HD pedigree in which both parents are affected by HD are shown. Progeny are shown as triangles for confidentiality and no HD diagnostic information is given to preserve the blind status of investigators in the Venezuelan Collaborative Group. AN1 and AN2 mark the positions of the allelic products from normal parental chromosomes. AE marks the range of PCR products from the *HD* chromosome. The PCR products from cosmids L191F1 and GUS72-2130 are loaded in lanes 29 and 30 and have 18 and 48 CAG repeats, respectively.

[0030] FIGURE 8. PCR analysis of the (CAG)<sub>n</sub> repeat in members of an American family with an individual homozygous for the major *HD* haplotype. Results of PCR analysis of members of an American family segregating the major HD haplotype. AN marks the range of normal alleles; AE marks the range of *HD* alleles. Lanes 1, 3, 4, 5, 7 and 8 represent PCR products from related *HD* heterozygotes. Lane 2 contains the PCR products from a member of the family homozygous for the same *HD* chromosome. Lane 6 contains PCR products from a normal individual. Pedigree relationships and affected status are not presented to preserve confidentiality. The PCR products from cosmids L191F1 and GUS72-2130 (which was derived from the individual represented in lane 2) are loaded in lanes 9 and 10 and have 18 and 48 CAG repeats, respectively.

[0031] FIGURES 9 and 10. PCR analysis of the (CAG)<sub>n</sub> repeat in two families with supposed new mutation causing HD. Results of PCR analysis of two families in which sporadic HD cases representing putative new mutants are shown. Individuals in each pedigree are numbered by generation (Roman numerals) and order in the pedigree. Triangles are used to protect confidentiality. Filled symbols indicate symptomatic individuals. The different chromosomes segregating in the pedigree have been distinguished by extensive typing with polymorphic markers in 4p16.3 and have been assigned arbitrary numbers shown above the gel lanes. The starred chromosomes (3 in Figure 9, 1 in Figure 10) represent the presumed *HD* chromosome. AN denotes the range of normal alleles; AE denotes the range of alleles present in affected individuals and in their unaffected relatives bearing the same chromosomes.

[0032] FIGURE 11. Comparison of (CAG)<sub>n</sub> Repeat Unit Number on Control and HD Chromosomes. Frequency distributions are shown for the number of (CAG)<sub>n</sub> repeat units observed on 425 HD chromosomes from 150 independent families, and from 545 control chromosomes.

[0033] FIGURE 12. Comparison of (CAG)<sub>n</sub> Repeat Unit Number on Maternally and Paternally Transmitted HD Chromosomes. Frequency distributions are shown for the 134 and 161 HD chromosomes from Figure 11 known to have been transmitted from the mother (Panel A) and father (Panel B), respectively. The two distributions differ significantly based on a t-test ( $t_{272,3}=5.34$ ,  $p < 0.0001$ ).

[0034] FIGURE 13. Comparison of (CAG)<sub>n</sub> Repeat Unit Number on HD Chromosomes from Three Large Families with Different HD Founders. Frequency distributions are shown for 75, 25 and 35 HD chromosomes from the Venezuelan HD family (Panel A) (Gusella, J.F., *et al.*, *Nature* 306:234-238 (1983); Wexler, N.S., *et al.*, *Nature* 326:194-197 (1987)), Family Z (Panel B) and Family D (Panel C) (Folstein, S.E., *et al.*, *Science* 229:776-779 (1985)), respectively. The Venezuelan distribution did not differ from the overall HD chromosome distribution in Figure 11 ( $t_{79,7}=1.58$ ,  $p < 0.12$ ). Both Family Z and Family D did produce distributions significantly different from the overall HD distribution ( $t_{42,2}=6.73$ ,  $p < 0.0001$  and  $t_{458}=2.90$ ,  $p < 0.004$ , respectively).

[0035] Figure 14. Relationship of (CAG)<sub>n</sub> Repeat Length in Parents and Corresponding Progeny. Repeat length on the HD chromosome in mothers (Panel A) or fathers (Panel B) is plotted against the repeat length in the corresponding offspring. A total of 25 maternal transmissions and 37 paternal transmissions were available for typing.

[0036] FIGURE 15. Amplification of the HD (CAG)<sub>n</sub> Repeat From Sperm and Lymphoblast DNA. DNA from sperm (S) and lymphoblasts (L) for 5 members (pairs 1-5) of the Venezuelan HD pedigree aged 24-30 were used for PCR amplification of the HD (CAG)<sub>n</sub> repeat. The lower band in each lane derives from the normal chromosome.

[0037] FIGURE 16. Relationship of Repeat Unit Length with Age of Onset. Age of onset was established for 234 diagnosed HD gene carriers and plotted against the repeat length observed on both the HD and normal chromosomes in the corresponding lymphoblast lines.

#### ***Detailed Description of the Invention***

[0038] In the following description, reference will be made to various methodologies known to those of skill in the art of molecular genetics and biology.

[0039] The IT15 gene described herein is a gene from the proximal portion of the 500 kb segment between human chromosome 4 markers D4S180 and D4S182. The huntingtin gene spans about 210 kb of DNA and encodes a previously undescribed protein of about 348 kDa. The huntingtin reading frame contains a polymorphic (CAG)<sub>n</sub> trinucleotide repeat with at least 17 alleles in the normal human population, where the repeat number varies from 11 to about 34 CAG copies in such alleles. This is the gene of the human chromosome that, as shown herein, suffers the presence of an unstable, expanded number of CAG trinucleotide repeats in Huntington's disease patients, such that the number of CAG repeats in the huntingtin gene increases to a range of 37 to at least 86 copies. These results are the basis of a conclusion that the huntingtin gene encodes a protein called "huntingtin," and that in such huntingtin gene the increase in the number of CAG repeats to a range of greater than about 37 repeats is the alteration that underlies the dominant phenotype of Huntington's disease. As used herein huntingtin gene is also called the Huntington's disease gene.

[0040] It is to be understood that the description below is applicable to any gene in which a CAG repeat within the gene is amplified in an aberrant manner resulting in a change in the regulation, localization, stability or translatability of the mRNA containing such amplified CAG repeat that is transcribed from such gene.

#### ***I. Cloning Of Huntingtin DNA And Expression Of Huntingtin Protein***

[0041] The identification of huntingtin DNA and protein as the altered gene in Huntington's disease patients is exemplified below. In addition to utilizing the exemplified methods and results for the identification of deletions of the huntingtin gene in Huntington's disease patients, and for the isolation of the native human huntingtin gene, the sequence information presented in Figure 4 represents a nucleic acid and protein sequence, that, when inserted into a linear or circular recombinant nucleic acid construct such as a vector, and used to transform a host cell, will provide copies of huntingtin DNA and huntingtin protein that are useful sources for the native huntingtin DNA and huntingtin protein for the methods of the invention. Such methods are known in the art and are briefly outlined below.

[0042] The process for genetically engineering the huntingtin coding sequence, for expression under a desired promoter, is facilitated through the cloning of genetic sequences which are capable of encoding such huntingtin protein. Such cloning technologies can utilize techniques known in the art for construction of a DNA sequence encoding the huntingtin protein, such as, for example, polymerase chain reaction technologies utilizing the huntingtin sequence disclosed herein to isolate the huntingtin gene anew, or an allele thereof that varies in the number of CAG repeats in such gene, or polynucleotide synthesis methods for constructing the nucleotide sequence using chemical methods. Expression of the cloned huntingtin DNA provides huntingtin protein.

[0043] As used herein, the term "genetic sequences" is intended to refer to a nucleic acid molecule of DNA or RNA, preferably DNA. Genetic sequences that are capable of being operably linked to DNA encoding huntingtin protein, so as to provide for its expression and maintenance in a host cell are obtained from a variety of sources, including commercial sources, genomic DNA, cDNA, synthetic DNA, and combinations thereof. Since the genetic code is universal, it is to be expected that any DNA encoding the huntingtin amino acid sequence of the invention will be useful to express huntingtin protein in any host, including prokaryotic (bacterial) hosts, eukaryotic hosts (plants, mammals (especially human), insects, yeast, and especially any cultured cell populations).

[0044] If it is desired to select anew a gene encoding huntingtin from a library that is thought to contain a huntingtin gene, such library can be screened and the desired gene sequence identified by any means which specifically selects for a sequence coding for the huntingtin gene or expressed huntingtin protein such as, for example, a) by hybridization (under stringent conditions for DNA:DNA hybridization) with an appropriate huntingtin DNA probe(s) containing a sequence specific for the DNA of this protein, such sequence being that provided in Figure 4 or a functional derivative thereof that is, a shortened form that is of sufficient length to identify a clone containing the huntingtin gene, or b) by hybridization-selected translational analysis in which native huntingtin mRNA which hybridizes to the clone in question is translated *in vitro* and the translation products are further characterized for the presence of a biological activity of huntingtin, or c) by immunoprecipitation of a translated huntingtin protein product from the host expressing the huntingtin protein.

[0045] When a human allele does not encode the identical sequence to that of Figure 4, it can be isolated and identified as being *huntingtin* DNA using the same techniques used herein, and especially PCR techniques to amplify the appropriate gene with primers based on the sequences disclosed herein. Many polymorphic probes useful in the fine localization of genes on chromosome 4 are known and available (see, for example, "ATCC/NIH Repository Catalogue of Human and Mouse DNA Probes and Libraries," fifth edition, 1991, pages 4-6. For example, a useful *D4S10* probe is clone designation pTV20 (ATCC 57605 and 57604); H5.52 (ATCC 61107 and 61106) and F5.53 (ATCC 61108).

[0046] Human chromosome 4-specific libraries are known in the art and available from the ATCC for the isolation of probes ("ATCC/NIH Repository Catalogue of Human and Mouse DNA Probes and Libraries," fifth edition, 1991, pages 72-73), for example, LL04NS01 and LL04NS02 (ATCC 57719 and ATCC57718) are useful for these purposes.

[0047] It is not necessary to utilize the exact vector constructs exemplified in the invention; equivalent vectors can be constructed using techniques known in the art. For example, the sequence of the *huntingtin* DNA is provided herein, (see Figure 4) and this sequence provides the specificity for the *huntingtin* gene; it is only necessary that a desired probe contain this sequence, or a portion thereof sufficient to provide a positive indication of the presence of the *huntingtin* gene.

[0048] *Huntingtin* genomic DNA may or may not include naturally occurring introns. Moreover, such genomic DNA can be obtained in association with the native *huntingtin* 5' promoter region of the gene sequences and/or with the native *huntingtin* 3' transcriptional termination region.

[0049] Such *huntingtin* genomic DNA can also be obtained in association with the genetic sequences which encode the 5' non-translated region of the *huntingtin* mRNA and/or with the genetic sequences which encode the *huntingtin* 3' non-translated region. To the extent that a host cell can recognize the transcriptional and/or translational regulatory signals associated with the expression of *huntingtin* mRNA and protein, then the 5' and/or 3' non-transcribed regions of the native *huntingtin* gene, and/or, the 5' and/or 3' non-translated regions of the *huntingtin* mRNA can be retained and employed for transcriptional and translational regulation.

[0050] Genomic DNA can be extracted and purified from any host cell, especially a human host cell possessing chromosome 4, by means well known in the art. Genomic DNA can be shortened by means known in the art, such as physical shearing or restriction digestion, to isolate the desired *huntingtin* gene from a chromosomal region that otherwise would contain more information than necessary for the utilization of the *huntingtin* gene in the hosts of the invention. For example, restriction digestion can be utilized to cleave the full-length sequence at a desired location. Alternatively, or in addition, nucleases that cleave from the 3'-end of a DNA molecule can be used to digest a certain sequence to a shortened form, the desired length then being identified and purified by polymerase chain reaction technologies, gel electrophoresis, and DNA sequencing. Such nucleases include, for example, Exonuclease III and *Ba*31. Other nucleases are well known in the art.

[0051] Alternatively, if it is known that a certain host cell population expresses *huntingtin* protein, then cDNA techniques known in the art can be utilized to synthesize a cDNA copy of the *huntingtin* mRNA present in such population.

[0052] For cloning the genomic or cDNA nucleic acid that encodes the amino acid sequence of the *huntingtin* protein into a vector, the DNA preparation can be ligated into an appropriate vector. The DNA sequence encoding *huntingtin* protein can be inserted into a DNA vector in accordance with conventional techniques, including blunt-ending or staggered-ending termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases.

[0053] When the *huntingtin* DNA coding sequence and an operably linked promoter are introduced into a recipient eukaryotic cell (preferably a human host cell) as a non-replicating, non-integrating, molecule, the expression of the encoded *huntingtin* protein can occur through the transient (nonstable) expression of the introduced sequence.

[0054] Preferably the coding sequence is introduced on a DNA molecule, such as a closed circular or linear molecule that is capable of autonomous replication. If integration into the host chromosome is desired, it is preferable to use a linear molecule. If stable maintenance of the *huntingtin* gene is desired on an extrachromosomal element, then it is preferable to use a circular plasmid form, with the appropriate plasmid element for autonomous replication in the desired host.

[0055] The desired gene construct, providing a gene coding for the *huntingtin* protein, and the necessary regulatory elements operably linked thereto, can be introduced into a desired host cells by transformation, transfection, or any method capable of providing the construct to the host cell. A marker gene for the detection of a host cell that has accepted the *huntingtin* DNA can be on the same vector as the *huntingtin* DNA or on a separate construct for co-transformation with the *huntingtin* coding sequence construct into the host cell. The nature of the vector will depend on the host organism.

[0056] Suitable selection markers will depend upon the host cell. For example, the marker can provide biocide resistance, e.g., resistance to antibiotics, or heavy metals, such as copper, or the like.

[0057] Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector can be recognized and selected from those recipient cells which do not contain the vector;

the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

[0058] When it is desired to use *S. cerevisiae* as a host for a shuttle vector, preferred *S. cerevisiae* yeast plasmids include those containing the 2-micron circle, etc., or their derivatives. Such plasmids are well known in the art and are commercially available.

[0059] Oligonucleotide probes specific for the *huntingtin* sequence can be used to identify clones to huntingtin and can be designed *de novo* from the knowledge of the amino acid sequence of the protein as provided herein in Figure 4 or from the knowledge of the nucleic acid sequence of the DNA encoding such protein as provided herein in Figure 4 or of a related protein. Alternatively, antibodies can be raised against the huntingtin protein and used to identify the presence of unique protein determinants in transformants that express the desired cloned protein.

[0060] A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a huntingtin protein if that nucleic acid contains expression control sequences which contain transcriptional regulatory information and such sequences are "operably linked" to the huntingtin nucleotide sequence which encode the huntingtin polypeptide.

[0061] An operable linkage is a linkage in which a sequence is connected to a regulatory sequence (or sequences) in such a way as to place expression of the sequence under the influence or control of the regulatory sequence. If the two DNA sequences are a coding sequence and a promoter region sequence linked to the 5' end of the coding sequence, they are operably linked if induction of promoter function results in the transcription of mRNA encoding the desired protein and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the expression regulatory sequences to direct the expression of the protein, antisense RNA, or (3) interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably linked to a DNA sequence if the promoter was capable of effecting transcription of that DNA sequence.

[0062] The precise nature of the regulatory regions needed for gene expression can vary between species or cell types, but shall in general include, as necessary, 5' non-transcribing and 5' non-translating (non-coding) sequences involved with initiation of transcription and translation respectively, such as the TATA box, capping sequence, CAAT sequence, and the like, with those elements necessary for the promoter sequence being provided by the promoters of the invention. Such transcriptional control sequences can also include enhancer sequences or upstream activator sequences, as desired.

[0063] The vectors of the invention can further comprise other operably linked regulatory elements such as DNA elements which confer antibiotic resistance, or origins of replication for maintenance of the vector in one or more host cells.

[0064] In another embodiment, especially for maintenance of the vectors of the invention in prokaryotic cells, or in yeast *S. cerevisiae* cells, the introduced sequence is incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors can be employed for this purpose. In *Bacillus* hosts, integration of the desired DNA can be necessary.

[0065] Expression of a protein in eukaryotic hosts such as a human cell requires the use of regulatory regions functional in such hosts. A wide variety of transcriptional and translational regulatory sequences can be employed, depending upon the nature of the host. Preferably, these regulatory signals are associated in their native state with a particular gene which is capable of a high level of expression in the specific host cell, such as a specific human tissue type. In eukaryotes, where transcription is not linked to translation, such control regions may or may not provide an initiator methionine (AUG) codon, depending on whether the cloned sequence contains such a methionine. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis in the host cell.

[0066] If desired, the non-transcribed and/or non-translated regions 3' to the sequence coding for the huntingtin protein can be obtained by the above-described cloning methods. The 3'-non-transcribed region of the native human *huntingtin* gene can be retained for its transcriptional termination regulatory sequence elements, or for those elements which direct polyadenylation in eukaryotic cells. Where the native expression control sequences signals do not function satisfactorily in a host cell, then sequences functional in the host cell can be substituted.

[0067] It may be desired to construct a fusion product that contains a partial coding sequence (usually at the amino terminal end) of a first protein or small peptide and a second coding sequence (partial or complete) of the huntingtin protein at the carboxyl end. The coding sequence of the first protein can, for example, function as a signal sequence for secretion of the huntingtin protein from the host cell. Such first protein can also provide for tissue targeting or localization of the huntingtin protein if it is to be made in one cell type in a multicellular organism and delivered to another cell type in the same organism. Such fusion protein sequences can be designed with or without specific protease sites such that a desired peptide sequence is amenable to subsequent removal.

[0068] The expressed huntingtin protein can be isolated and purified from the medium of the host in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis, or the like. For example, affinity purification with anti-huntingtin antibody can be used. A protein having the amino acid sequence shown in Figure 3 can be made, or a shortened peptide of this sequence can be made, and used to raise

antibodies using methods well known in the art. These antibodies can be used to affinity purify or quantitate huntingtin protein from any desired source.

[0069] If it is necessary to extract huntingtin protein from the intracellular regions of the host cells, the host cells can be collected by centrifugation, or with suitable buffers, lysed, and the protein isolated by column chromatography, for example, on DEAE-cellulose, phosphocellulose, polyribocytidylic acid-agarose, hydroxyapatite or by electrophoresis or immunoprecipitation.

## II. Use Of Huntingtin For Diagnostic And Treatment Purposes

[0070] It is to be understood that although the following discussion is specifically directed to human patients, the teachings are also applicable to any animal that expresses huntingtin and in which alteration of huntingtin, especially the amplification of CAG repeat copy number, leads to a defect in huntingtin gene (structure or function) or huntingtin protein (structure or function or expression), such that clinical manifestations such as those seen in Huntington's disease patients are found.

[0071] It is also to be understood that the methods referred to herein are applicable to any patient suspected of developing/having Huntington's disease, whether such condition is manifest at a young age or at a more advanced age in the patient's life. It is also to be understood that the term "patient" does not imply that symptoms are present, and patient includes any individual it is desired to examine or treat using the methods of the invention.

[0072] The diagnostic and screening methods of the invention are especially useful for a patient suspected of being at risk for developing Huntington's disease based on family history, or a patient in which it is desired to diagnose or eliminate the presence of the Huntington's disease condition as a causative agent behind a patient's symptoms.

[0073] It is to be understood that to the extent that a patient's symptoms arise due to the alteration of the CAG repeat copy numbers in the *huntingtin* gene, even without a diagnosis of Huntington's disease, the methods of the invention can identify the same as the underlying basis for such condition.

[0074] According to the invention, presymptomatic screening of an individual in need of such screening for their likelihood of developing Huntington's disease is now possible using DNA encoding the huntingtin gene of the invention, and specifically, DNA having the sequence of the normal human huntingtin gene. The screening method of the invention allows a presymptomatic diagnosis, including prenatal diagnosis, of the presence of an aberrant *huntingtin* gene in such individuals, and thus an opinion concerning the likelihood that such individual would develop or has developed Huntington's disease or symptoms thereof. This is especially valuable for the identification of carriers of altered huntingtin gene alleles where such alleles possess an increased number of CAG repeats in their huntingtin gene, for example, from individuals with a family history of Huntington's disease. Especially useful for the determination of the number of CAG repeats in the patient's *huntingtin* gene is the use of PCR to amplify such region or DNA blotting techniques.

[0075] For example, in the method of screening, a tissue sample would be taken from such individual, and screened for (1) the presence of the 'normal' human *huntingtin* gene, especially for the presence of a "normal" range of 11-34 CAG copies in such gene. The human *huntingtin* gene can be characterized based upon, for example, detection of restriction digestion patterns in 'normal' versus the patient's DNA, including RFLP analysis, using DNA probes prepared against the *huntingtin* sequence (or a functional fragment thereof) taught in the invention. Similarly, huntingtin mRNA can be characterized and compared to normal huntingtin mRNA (a) levels and/or (b) size as found in a human population not at risk of developing Huntington's disease using similar probes. Lastly, huntingtin protein can be (a) detected and/or (b) quantitated using a biological assay for huntingtin, for example, using an immunological assay and anti-huntingtin antibodies. When assaying huntingtin protein, the immunological assay is preferred for its speed. Methods of making antibody against the huntingtin are well known in the art.

[0076] An (1) aberrant *huntingtin* DNA size pattern, such as an aberrant *huntingtin* RFLP, and/or (2) aberrant huntingtin mRNA sizes or levels and/or (3) aberrant huntingtin protein levels would indicate that the patient has developed or is at risk for developing a huntingtin-associated symptom such as a symptom associated with Huntington's disease.

[0077] The screening and diagnostic methods of the invention do not require that the entire huntingtin DNA coding sequence be used for the probe. Rather, it is only necessary to use a fragment or length of nucleic acid that is sufficient to detect the presence of the huntingtin gene in a DNA preparation from a normal or affected individual, the absence of such gene, or an altered physical property of such gene (such as a change in electrophoretic migration pattern).

[0078] Prenatal diagnosis can be performed when desired, using any known method to obtain fetal cells, including amniocentesis, chorionic villous sampling (CVS), and fetoscopy. Prenatal chromosome analysis can be used to determine if the portion of chromosome 4 possessing the normal *huntingtin* gene is present in a heterozygous state, and PCR amplification or DNA blotting utilized for estimating the size of the CAG repeat in the *huntingtin* gene.

[0079] The huntingtin DNA can be synthesized, especially, the CAG repeat region can be amplified and, if desired, labeled with a radioactive or nonradioactive reporter group, using techniques known in the art (for example, see Eckstein, F., Ed., *Oligonucleotides and Analogues: A Practical Approach*, IRS Press at Oxford University Press, New York,

1992); and Kricka, L.J., Ed., *Nonisotopic DNA Probe Techniques*, Academic Press, San Diego, (1992)).

5 [0080] Functional *huntingtin* DNA may be used in the manufacture of a medicament for treating Huntington's disease in a patient in need of such treatment, the medicament being for administration to the cells of such patient, preferably prior to such symptomatic state that indicates the death of many of the patient's neuronal cells which it is desired to target with the method of the invention. The replacement *huntingtin* DNA is provided in a manner and amount that permits the expression of the *huntingtin* protein provided by such gene, for a time and in a quantity sufficient to treat such patient. Many vector systems are known in the art to provide such delivery to human patients in need of a gene or protein missing from the cell. For example, adenovirus or retrovirus systems can be used, especially modified retrovirus systems and especially herpes simplex virus systems. Such methods are provided for, in, for example, the teachings of Breakefield, X.A. *et al.*, *The New Biologist* 3:203-218 (1991); Huang, Q. *et al.*, *Experimental Neurology* 115:303-316 (1992), WO93/03743 and WO90/09441. Methods of antisense strategies are known in the art (see, for example, *Antisense Strategies*, Baserga, R. *et al.*, Eds., *Annals of the New York Academy of Sciences*, volume 660, 10 1992).

15 [0081] A gene encoding an expressible sequence that transcribes *huntingtin* antisense RNA may be used in the manufacture of a medicament for treating Huntington's disease in a patient in need of such treatment, the medicament being for administration to the cells of such patient, preferably prior to such symptomatic state that indicates the death of many of the patient's neuronal cells which it is desired to target with the method of the invention. The replacement *huntingtin* antisense RNA gene is provided in a manner and amount that permits the expression of the antisense RNA provided by such gene, for a time and in a quantity sufficient to treat such patient, and especially in an amount to inhibit 20 translation of the aberrant *huntingtin* mRNA that is being expressed in the cells of such patient. As above, many vector systems are known in the art to provide such delivery to human patients in need of a gene or protein which is altered in the patients' cells. For example, adenovirus or retrovirus systems can be used, especially modified retrovirus systems and especially herpes simplex virus systems. Such methods are provided for, in, for example, the teachings of Breakefield, X.A. *et al.*, *The New Biologist* 3:203-218 (1991); Huang, Q. *et al.*, *Experimental Neurology* 115:303-316 (1992), 25 WO93/03743 and WO90/09441.

[0082] Delivery of a DNA sequence encoding a functional *huntingtin* protein, such as the amino acid encoding sequence of Figure 4, will effectively replace the altered *huntingtin* gene of the invention, and inhibit, and/or stop and/or regress the symptoms that are the result of the interference to *huntingtin* gene expression due to an increased number of CAG repeats, such as 37 to 86 repeats in the *huntingtin* gene as compared to the 11-34 CAG repeats found in human 30 populations not at risk for developing Huntington's disease.

[0083] Because Huntington's disease is characterized by a loss of neurons that is most severe in the caudate and putamen regions of the brain, the method of treatment of the invention is most effective when the replacement *huntingtin* gene is provided to the patient early in the course of the disease, prior to the loss of many neurons due to cell death. For that reason, presymptomatic screening methods according to the invention are important in identifying those 35 individuals in need of treatment by the method of the invention, and such treatment preferably is provided while such individual is presymptomatic.

[0084] An antagonist to the aberrant *huntingtin* protein may be used in the manufacture of a medicament for treating Huntington's disease in a patient in need of such treatment, the medicament being for administration in the cells of such patient.

40 [0085] Although the use is specifically described for DNA-DNA probes, it is to be understood that RNA possessing the same sequence information as the DNA of the invention can be used when desired.

[0086] For diagnostic assays, *huntingtin* antibodies are useful for quantitating and evaluating levels of *huntingtin* protein, and are especially useful in immunoassays and diagnostic kits.

45 [0087] In another embodiment, the present invention relates to an antibody having binding affinity to an *huntingtin* polypeptide, or a binding fragment thereof. In a preferred embodiment, the polypeptide has the amino acid sequence set forth in SEQ ID NO:6, or mutant or species variation thereof, or at least 7 contiguous amino acids thereof (preferably, at least 10, 15, 20, or 30 contiguous amino acids thereof). Those which bind selectively to *huntingtin* would be chosen for use in methods which could include, but should not be limited to, the analysis of altered *huntingtin* expression in tissue containing *huntingtin*.

50 [0088] The antibodies of the present invention include monoclonal and polyclonal antibodies, as well fragments of these antibodies. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')<sub>2</sub> fragment; the Fab' fragments, and the Fab fragments.

55 [0089] Of special interest to the present invention are antibodies to *huntingtin* (or their functional derivatives) which are produced in humans, or are "humanized" (i.e. non-immunogenic in a human) by recombinant or other technology. Humanized antibodies may be produced, for example by replacing an immunogenic portion of an antibody with a corresponding, but non-immunogenic portion (i.e. chimeric antibodies) (Robinson, R.R. *et al.*, International Patent Publication PCT/US86/02269; Akira, K. *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Ap-

plication 171,496; Morrison, S.L. *et al.*, European Patent Application 173,494; Neuberger, M.S. *et al.*, PCT Application WO 86/01533; Cabilly, S. *et al.*, European Patent Application 125,023; Better, M. *et al.*, *Science* 240:1041-1043 (1988); Liu, A.Y. *et al.*, *Proc. Natl. Acad. Sci. USA* 84:3439-3443 (1987); Liu, A.Y. *et al.*, *J. Immunol.* 139:3521-3526 (1987); Sun, L.K. *et al.*, *Proc. Natl. Acad. Sci. USA* 84:214-218 (1987); Nishimura, Y. *et al.*, *Canc. Res.* 47:999-1005 (1987); Wood, C.R. *et al.*, *Nature* 314:446-449 (1985); Shaw *et al.*, *J. Natl. Cancer Inst.* 80:1553-1559 (1988). General reviews of "humanized" chimeric antibodies are provided by Morrison, S.L. (*Science*, 229:1202-1207 (1985)) and by Oi, V.T. *et al.*, *BioTechniques* 4:214 (1986). Suitable "humanized" antibodies can be alternatively produced by CDR or CEA substitution (Jones, P.T. *et al.*, *Nature* 321:552-525 (1986); Verhoeven *et al.*, *Science* 239:1534 (1988); Beidler, C.B. *et al.*, *J. Immunol.* 141:4053-4060 (1988)).

5 [0090] In another embodiment, the present invention relates to a hybridoma which produces the above-described monoclonal antibody, or binding fragment thereof. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

10 [0091] In general, techniques for preparing monoclonal antibodies and hybridomas are well known in the art (Campbell, *"Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology,"* Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth *et al.*, *J. Immunol. Methods* 35:1-21 (1980)).

15 [0092] Any animal (mouse, rabbit, and the like) which is known to produce antibodies can be immunized with the selected polypeptide. Methods for immunization are well known in the art. Such methods include subcutaneous or interperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of polypeptide used for immunization will vary based on the animal which is immunized, the antigenicity of the polypeptide and the site of injection.

20 [0093] The polypeptide may be modified or administered in an adjuvant in order to increase the peptide antigenicity. Methods of increasing the antigenicity of a polypeptide are well known in the art. Such procedures include coupling the antigen with a heterologous protein (such as globulin or  $\beta$ -galactosidase) or through the inclusion of an adjuvant during immunization.

25 [0094] For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells.

30 [0095] Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz *et al.*, *Exp. Cell Res.* 175:109-124 (1988)).

35 [0096] Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology, supra* (1984)).

40 [0097] For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

45 [0098] In another embodiment of the present invention, the above-described antibodies are detectably labeled. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, and the like), enzymatic labels (such as horse radish peroxidase, alkaline phosphatase, and the like) fluorescent labels (such as FITC or rhodamine, and the like), paramagnetic atoms, and the like. Procedures for accomplishing such labeling are well-known in the art, for example, see (Sternberger *et al.*, *J. Histochem. Cytochem.* 18:315 (1970); Bayer *et al.*, *Meth. Enzym.* 62:308 (1979); Engval *et al.*, *Immunol.* 109:129 (1972); Goding, *J. Immunol. Meth.* 13:215 (1976)). The labeled antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues which express a specific peptide.

50 [0099] The above-described antibodies may be immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir *et al.*, *"Handbook of Experimental Immunology"* 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby *et al.*, *Meth. Enzym.* 34 Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays as well as in immunochromatography.

55 [0100] Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques, methods and kits disclosed above with regard to antibodies, to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed anti-peptide peptides, for example see Hurby *et al.*, "Application of Synthetic Peptides: Antisense Peptides", In *Synthetic Peptides, A User's Guide*, W.H. Freeman, NY, pp. 289-307 (1992), and Kaspaczak *et al.*, *Biochemistry* 28:9230-8 (1989).

60 [0101] Anti-peptide peptides can be generated in one of two fashions. First, the anti-peptide peptides can be generated by replacing the basic amino acid residues found in the huntingtin peptide sequence with acidic residues, while maintaining hydrophobic and uncharged polar groups. For example, lysine, arginine, and/or histidine residues are replaced with aspartic acid or glutamic acid and glutamic acid residues are replaced by lysine, arginine or histidine.

65 [0102] The manner and method of carrying out the present invention can be more fully understood by those of skill

by reference to the following examples.

**Examples**

5 [0103] The gene causing Huntington's disease has been mapped in 4p16.3 but has previously eluded identification. The invention uses haplotype analysis of linkage disequilibrium to spotlight a small segment of 4p16.3 as the likely location of the defect. A new gene, *huntingtin* (*HT15*), isolated using cloned "trapped" exons from a cosmid contig of the target area contains a polymorphic trinucleotide repeat that is expanded and unstable on HD chromosomes. A (CAG)<sub>n</sub> repeat longer than the normal range of about 11 to about 34 copies was observed on HD chromosomes from  
10 all 75 disease families examined, comprising a wide range of ethnic backgrounds and 4p16.3 haplotypes. The (CAG)<sub>n</sub> repeat, which varies from 37 to at least 86 copies on HD chromosomes appears to be located within the coding sequence of a predicted about 348 kDa protein that is widely expressed but unrelated to any known gene. Thus, the Huntington's disease mutation involves an unstable DNA segment, similar to those described in fragile X syndrome and myotonic dystrophy, acting in the context of a novel 4p16.3 gene to produce a dominant phenotype.

15 [0104] The following protocols and experimental details are referenced in the examples that follow.

[0105] *HD Cell Lines.* Lymphoblast cell lines from HD families of varied ethnic backgrounds used for genetic linkage and disequilibrium studies (Conneally *et al.*, *Genomics* 5:304-308 (1989); MacDonald *et al.*, *Nature Genet.* 1:99-103 (1992)) have been established (Anderson and Gusella, *In Vitro* 20:856-858 (1984)) in the Molecular Neurogenetics Unit, Massachusetts General Hospital, over the past 13 years. The Venezuelan HD pedigree is an extended kindred of over 10,000 members in which all affected individuals have inherited the *HD* gene from a common founder (Gusella *et al.*, *Nature* 306:234-238 (1983); Gusella *et al.*, *Science* 225:1320-1326 (1984); Wexler *et al.*, *Nature* 326:194-197 (1987)).

20 [0106] *DNA/RNA Blotting.* DNA was prepared from cultured cells and DNA blots prepared and hybridized as described (Gusella *et al.*, *Proc. Natl. Acad. Sci. USA* 76:5239-5243 (1979); Gusella *et al.*, *Nature* 306:234-238 (1983)). RNA was prepared and Northern blotting performed as described in Taylor *et al.*, *Nature Genet.* 3:223-227 (1992).

25 [0107] *Construction of Cosmid Contig.* The initial construction of the cosmid contig was by chromosome walking from cosmids L19 and BJ56 (Allitto *et al.*, *Genomics* 9:104-112 (1991); Lin *et al.*, *Somat. Cell Mol. Genet.* 17:481-488 (1991)). Two libraries were employed, a collection of Alu-positive cosmids from the reduced cell hybrid H39-8C10 (Whaley *et al.*, *Som. Cell Mol. Genet.* 17:83-91 (1991)) and an arrayed flow-sorted chromosome 4 cosmid library (NM87545) provided by the Los Alamos National Laboratory. Walking was accomplished by hybridization of whole cosmid DNA, using suppression of repetitive and vector sequences, to robot-generated high density filter grids (Nizetic, D. *et al.*, *Proc. Natl. Acad. Sci. USA* 88:3233-3237 (1991); Lehrach, H. *et al.*, in *Genome Analysis: Genetic and Physical Mapping, Volume 1*, Davies, K.E. *et al.*, Ed., Cold Spring Harbor Laboratory Press, 1991, pp. 39-81). Cosmids L1C2, L69F7, L228B6 and L83D3 were first identified by hybridization of YAC clone YGA2 to the same arrayed library (Bates *et al.*, *Nature Genet.* 1:180-187 (1992); Baxendale *et al.*, *Nucleic Acids Res.* 19:6651 (1991)). *HD* cosmid GUS72-2130 was isolated by standard screening of a GUS72 cosmid library using a single-copy probe. Cosmid overlaps were confirmed by a combination of clone-to-clone and clone-to-genomic hybridizations, single-copy probe hybridizations and restriction mapping.

30 [0108] *cDNA Isolation and Characterization.* Exon probes were isolated and cloned as described (Buckler *et al.*, *Proc. Natl. Acad. Sci. USA* 88:4005-4009 (1991)). Exon probes and cDNAs were used to screen human 1ambdaZAPII cDNA libraries constructed from adult frontal cortex, fetal brain, adenovirus transformed retinal cell line RCA, and liver RNA. cDNA clones, PCR products and trapped exons were sequenced as described (Sanger *et al.*, *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)). Direct cosmid sequencing was performed as described (McClatchey *et al.*, *Hum. Mol. Genet.* 1:521-527 (1992)). Database searches were performed using the BLAST network service of National Center for Biotechnology Information (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)).

35 [0109] *PCR Assay of the (CAG)<sub>n</sub> Repeat.* Genomic primers (SEQ ID NO:3 and SEQ ID NO:4) flanking the (CAG)<sub>n</sub> repeat are:

50 5' ATG AAG GCC TTC GAG TCC CTC AAG TCC TTC 3'

and

55 5' AAA CTC ACG GTC GGT GCA GCG GCT CCT CAG 3'.

PCR amplification was performed in a reaction volume of 25  $\mu$ l using 50 ng of genomic DNA, 5  $\mu$ g of each primer, 10

5 mM Tris, pH 8.3, 5mM KCl, 2mM MgCl<sub>2</sub>, 200  $\mu$ M dNTPs, 10% DMSO, 0.1 unit Perfectmatch (Stratagene), 2.5  $\mu$ Ci <sup>32</sup>P-dCTP (Amersham) and 1.25 units Taq polymerase (Boehringer Mannheim). After heating to 94°C for 1.5 minutes, the reaction mix was cycled according to the following program: 40 X [1'@94°C;1'@60°C;2'@72°C]. 5  $\mu$ l of each PCR reaction was diluted with an equal volume of 95 % formamide loading dye and heat denatured for 2 min. at 95°C. The products were resolved on 5% denaturing polyacrylamide gels. The PCR product from this reaction using cosmid L191F1 (CAG<sub>18</sub>) as template was 247 bp. Allele sizes were estimated relative to a DNA sequencing ladder, the PCR products from sequenced cosmids, and the invariant background bands often present on the gel. Estimates of allelic variation were obtained by typing unrelated individuals of largely Western European ancestry, and normal parents of affected HD individuals from various pedigrees.

10 [0110] *Typing of HD and normal chromosomes in Examples 5-8.* HD chromosomes were derived from symptomatic individuals and "at risk" individuals known to be gene carriers by linkage marker analysis. All HD chromosomes were from members of well-characterized HD families of varied ethnic backgrounds used previously for genetic linkage and disequilibrium studies (MacDonald, M.E., et al., *Nature Genet.* 1:99-103 (1992); Conneally, P.M., et al., *Genomics* 5: 304-308 (1989)). Three of the 150 families used were large pedigrees, each descended from a single founder. The 15 large Venezuelan HD pedigree is an extended kindred of over 13,000 members from which we typed 75 HD chromosomes (Gusella, J.F., et al., *Nature* 306:234-238 (1983); Wexler, N.S., et al., *Nature* 326:194-197 (1987)). Two other large families that have been described previously as Family Z and Family D, provided 25 and 35 HD chromosomes, respectively (Folstein, S.E., et al., *Science* 229:776-779 (1985)). Normal chromosomes were taken from married-ins in the HD families and from unrelated normal individuals from non-HD families. The DNA tested for all individuals 20 except four was prepared from lymphoblastoid cell lines or fresh blood (Gusella, J.F., et al., *Nature* 306:234-238 (1983); Anderson and Gusella, *In Vitro* 20:856-858 (1984)). In the exceptional cases, DNA was prepared from frozen cerebellum. No difference in the characteristics of the PCR products were observed between lymphoblastoid, fresh blood, or brain DNAs. For five members of the Venezuelan pedigree aged 24-30, we also prepared DNA by extracting pelleted sperm from semen samples. The length of the HD gene (CAG)<sub>n</sub> repeat for all DNAs was assessed using polymerase 25 chain reaction amplification.

25 [0111] *Statistical analysis as set forth in Examples 5-8.* Associations between repeat lengths and onset age were assessed by Pearson correlation coefficient and by multivariate regression to assess higher order associations. Comparisons of the distributions of repeat length for all HD chromosomes and those for individual families were made by analysis of variance and t-test contrasts between groups. The 95 % confidence bands were computed around the 30 regression line utilizing the general linear models procedure of SAS (SAS Institute Inc., SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2 (SAS Institute Inc., Cary, N.C., pp. 846, 1989)).

### Example 1

35 *Application of Exon Amplification to Obtain Trapped Cloned Exons*

40 [0112] The HD candidate region defined by discrete recombination events in well-characterized families spans 2.2 Mb between *D4S10* and *D4S98* as shown in Figure 1. The 500 kb segment between *D4S180* and *D4S182* displays the strongest linkage disequilibrium with HD, with about 1/3 of disease chromosomes sharing a common haplotype, anchored by multi-allele polymorphisms at *D4S127* and *D4S95* (MacDonald et al., *Nature Genet.* 1:99-103 (1992)). Sixty-four overlapping cosmids spanning about 480 kb from *D4S180* to a location between *D4S95* and *D4S182* have been isolated by a combination of information from YAC (Baxendale et al., *Nucleic Acids Res.* 19:6651 (1991)) and cosmid probe hybridization to high density filter grids of a chromosome 4 specific library, as well as additional libraries covering this region. Sixteen of these cosmids providing the complete contig are shown in Figure 1. We have previously 45 used exon amplification to identify *ADDA*, the  $\alpha$ -adducin locus, *IT10C3*, a novel putative transporter gene, and *IT11*, a novel G protein-coupled receptor kinase gene in the region distal to *D4S127* (Figure 1).

50 [0113] We have now applied the exon amplification technique to cosmids from the region of the contig proximal to *D4S127*. This procedure produces "trapped" exon clones, which can represent single exons, or multiple exons spliced together and is an efficient method of obtaining probes for screening cDNA libraries. Individual cosmids were processed, yielding 9 exon clones in the region from cosmids L134B9 to L181B10.

55 [0114] Two non-overlapping cDNAs were initially isolated using exon probes. *IT15A* was obtained by screening a transformed adult retinal cell cDNA library with exon clone DL118F5-U. *IT16A* was isolated by screening an adult frontal cortex cDNA library with a pool of three exon clones, DL83D3-8, DL83D3-1, and DL228B6-3. By Northern blot analysis, we discovered that *IT15A* and *IT16A* are in fact different portions of the same large approximately 10-11 kb transcript. Figure 2 shows an example of a Northern blot containing RNA from lymphoblastoid cell lines representing a normal individual and 2 independent homozygotes for HD chromosomes of different haplotypes. The same approximately 10-11 kb transcript was also detected in RNA from a variety of human tissues (liver, spleen, kidney, muscle and various regions of adult brain).

[0115] IT15A and IT16A were used to "walk" in a number of human tissue cDNA libraries in order to obtain the full-length transcript. Figure 3 shows a representation of 5 cDNA clones which define the IT15 transcript, under a schematic of the composite sequence derived as described in the legend. Figure 3 also displays the locations on the composite sequence of the 9 trapped exon clones.

[0116] The composite sequence of IT15, containing the entire predicted coding sequence, spans 10,366 bases including a tail of 18 A's as shown in Figure 4. An open reading frame of 9,432 bases begins with a potential initiator methionine codon at base 316, located in the context of an optimal translation initiation sequence. An in-frame stop codon is located 240 bases upstream from this site. The protein product of IT15 is predicted to be a 348 kDa protein containing 3,144 amino acids. Although the first Met codon in the long open reading frame has been chosen as the probably initiator codon, we cannot exclude that translation does not actually begin at a more 3' Met codon, producing a smaller protein.

#### Example 2

15 *Polymorphic Variation of the (CAG)<sub>n</sub> Trinucleotide Repeat*

[0117] Near its 5' end, the IT15 sequence contains 21 copies of the triplet CAG, encoding glutamine (Figure 5). When this sequence was compared with genomic sequences that are known to surround simple sequence repeats (SSRs) in 4p16.3, it was found that normal cosmid L191F1 had 18 copies of the triplet indicating that the (CAG)<sub>n</sub> repeat is polymorphic (Figure 5). Primers from the genomic sequence flanking the repeat were chosen to establish a PCR assay for this variation. In the normal population, this SSR polymorphism displays at least 17 discrete alleles (Table 1) ranging from about 11 to about 34 repeat units. Ninety-eight percent of the 173 normal chromosomes tested contained repeat lengths between 11 and 24 repeats. Two chromosomes were detected in the 25-30 repeat range and 2 normal chromosomes had 33 and 34 repeats respectively. The overall heterozygosity on normal chromosome was 80%. Based on sequence analysis of three clones, it appears that the variation is based entirely on the (CAG)<sub>n</sub>, but the potential for variation of the smaller downstream (CCG)<sub>7</sub>, which is also included in the PCR product, is also present.

#### Example 3

30 *Instability of the Trinucleotide Repeat on HD chromosomes*

[0118] Sequence analysis of cosmid GUS72-2130, derived from a chromosome with the major *HD* haplotype (see below), revealed 48 copies of the trinucleotide repeat, far greater than the largest normal allele (Figure 5). When the PCR assay was applied to *HD* chromosomes, a pattern strikingly different from the normal variation was observed.

35 *HD* heterozygotes contained one discrete allelic product in the normal size range, and one PCR product of much larger size, suggesting that the (CAG)<sub>n</sub> repeat on *HD* chromosomes is expanded relative to normal chromosomes.

[0119] Figure 6 shows the patterns observed when the PCR assay was performed on lymphoblast DNA from a selected nuclear family in a large Venezuelan *HD* kindred. In this family, DNA marker analysis has shown previously that the *HD* chromosome was transmitted from the father (lane 2) to seven children (lanes 3, 5, 6, 7, 8, 10 and 11).

40 The three normal chromosomes present in this mating yielded a PCR product in the normal size range (AN1, AN2, AN3) that was inherited in a Mendelian fashion. The *HD* chromosome in the father yielded a diffuse, "fuzzy"-appearing PCR product slightly smaller than the 48 repeat product of the non-Venezuelan *HD* cosmid. Except for the DNA in lane 5 which did not PCR amplify and in lane 11 which displayed only a single normal allele, each of the affected children's DNAs yielded a fuzzy PCR product of a different size (AE), indicating instability of the *HD* chromosome (CAG)<sub>n</sub> repeat.

45 Lane 6 contained an *HD*-specific product slightly smaller than or equal to that of the father's DNA. Lanes 3, 7, 10 and 8, respectively, contained *HD*-specific PCR products of progressively larger size. The absence of an *HD*-specific PCR product in lane 11 suggested that this child's DNA possessed a (CAG)<sub>n</sub> repeat that was too long to amplify efficiently. This was verified by Southern blot analysis in which the expanded *HD* allele was easily detected and estimated to contain up to 100 copies of the repeat. Notably, this child had juvenile onset of *HD* at the very early age of 2 years.

50 The onset of *HD* in the father was in his early 40s, typical of most adult *HD* patients in this population. The onset ages of children represented by lanes 3, 7, 10 and 8 were 26, 25, 14 and 11 years, respectively, suggesting a rough correlation between age at onset of *HD* and the length of the (CAG)<sub>n</sub> repeat on the *HD* chromosome. In keeping with this trend, the offspring represented in lane 6 with the fewest repeats remained asymptomatic when last examined at age of 30.

[0120] Figure 7 shows PCR analysis for a second sibship from the Venezuelan pedigree in which both parents are 55 *HD* heterozygotes carrying the same *HD* chromosome based on DNA marker studies. Several of the offspring are *HD* homozygotes (lanes 6+7, 10+11, 13+14, 17+18, 23+24) as reported previously (Wexler *et al.*, *Nature* 326:194-197 (1987)). Each parent's DNA contained one allele in the normal range (AN1, AN2) which was transmitted in a Mendelian fashion. The *HD*-specific products (AE) from the DNA of both parents and children were all much larger than the normal

5 allelic products and also showed extensive variation in mean size. A neurologic diagnosis for the offspring in this pedigree was not provided to maintain the blind status of investigators involved in the ongoing Venezuela HD project, although age of onset again appears to parallel repeat length. Paired samples under many of the individual symbols represent independent lymphoblast lines initiated at least one year apart. The variance between paired samples was not as great as between the different individuals, suggesting that the major differences in size of the PCR products resulted from meiotic transmission. Of special note is the result obtained in lanes 13 and 14. This *HD* homozygote's DNA yielded one PCR product larger and one smaller than the *HD*-specific PCR products of both parents.

10 [0121] To date, we have tested 75 independent HD families, representing all different reported in MacDonald *et al.*, *Nature Genet.* 1:99-103 (1992)) and a wide range of ethnic backgrounds. In all 75 cases, a PCR product larger than the normal size range was produced from the *HD* chromosome. The sizes of the *HD*-specific products ranged from 42 repeat copies to more than 66 copies, with a few individuals failing to yield a product because of the extreme length of the repeat. In these cases, Southern blot analysis revealed an increase in the length of an EcoRI fragment with the largest allele approximating 100 copies of the repeat. Figure 8 shows the variation detected in members of an American family of Irish ancestry in which the major *HD* haplotype is segregating. Cosmid GUS72-2130 was cloned from the *HD* homozygous individual whose DNA was amplified in lane 2. As was observed in the Venezuelan HD pedigree (Figures 6 and 7), which segregates the disorder with a different 4p16.3 haplotype, the *HD*-specific PCR products for this family display considerable size variation.

#### Example 4

##### New Mutations to HD

20 [0122] The mutation rate in HD has been reported to be very low. To test whether the expansion of the (CAG)<sub>n</sub> repeat is the mechanism by which new *HD* mutations occur, two pedigrees with sporadic cases of HD have been examined in which intensive searching failed to reveal a family history of the disorder. In these cases, pedigree information sufficient to identify the same chromosomes in both the affected individual and unaffected relatives was gathered. Figures 9 and 10 show the results of PCR analysis of the (CAG)<sub>n</sub> repeat in these families. The chromosomes in each family were assigned an arbitrary number based on typing for a large number of RFLP and SSR markers in 4p16.3 defining distinct haplotypes and the presumed *HD* chromosome is starred.

25 [0123] In family #1, HD first appeared in individual II-3 who transmitted the disorder to III-1 along with chromosome 3\*. This same chromosome was present in II-2, an elderly unaffected individual. PCR analysis revealed that chromosome 3\* from II-2 produced a PCR product at the extreme high end of the normal range (about 36 CAG copies). However, the (CAG)<sub>n</sub> repeat on the same chromosome in II-3 and III-1 had undergone sequential expansions to about 44 and about 46 copies, respectively. A similar result was obtained in Family #2, where the presumed *HD* mutant III-35 2 had a considerably expanded repeat relative to the same chromosome in II-1 and III-1 (about 49 vs. about 33 CAG copies). In both family #1 and family #2, the ultimate *HD* chromosome displays the marker haplotype characteristic of 1/3 of all *HD* chromosomes, suggesting that this haplotype may be predisposed to undergoing repeat expansion.

##### Discussion

40 [0124] The discovery of an expanded, unstable trinucleotide repeat on *HD* chromosomes within the *IT15* gene is the basis for utilizing this gene as the *HD* gene of the invention. These results are consistent with the interpretation that HD constitutes the latest example of a mutational mechanism that may prove quite common in human genetic disease. Elongation of a trinucleotide repeat sequence has been implicated previously as the cause of three quite different 45 human disorders, the fragile X syndrome, myotonic dystrophy and spino-bulbar muscular atrophy. The initial observations of repeat expansion in HD indicate that this phenomenon shares features in common with each of these disorders.

45 [0125] In the fragile X syndrome, expression of a constellation of symptoms that includes mental retardation and a fragile site at Xq27.3 is associated with expansion of a (CGG)<sub>n</sub> repeat thought to be in the 5' untranslated region of the *FMR1* gene (Fu *et al.*, *Cell* 67:1047-1058 (1991); Kremer *et al.*, *Science* 252:1711-1714 (1991); Verkerk *et al.*, *Cell* 65:904-914 (1991)). In myotonic dystrophy, a dominant disorder involving muscle weakness with myotonia that typically 50 present in early adulthood, the unstable trinucleotide repeat, (CTG)<sub>n</sub>, is located in the 3' untranslated region of the myotonin protein kinase gene (Aslanidis *et al.*, *Nature* 355:548-551 (1992); Brook *et al.*, *Cell* 68:799-808 (1992); Buxton *et al.*, *Nature* 355:547-548 (1992); Fu *et al.*, *Science* 255:1256-1259 (1992); Harley *et al.*, *Lancet* 339:1125-1128 (1992); Mahadevan *et al.*, *Science* 255:1253-1255 (1992)). The unstable (CAG)<sub>n</sub> repeat in HD may be within the coding 55 sequence of the *IT15* gene, a feature shared with spino-bulbar muscular atrophy, an X-linked recessive adult-onset disorder of the motor neurons caused by expansion of a (CAG)<sub>n</sub> repeat in the coding sequence of the androgen receptor gene (LaSpada *et al.*, *Nature* 352:77-79 (1991)). The repeat length in both the fragile X syndrome and myotonic dystrophy tends to increase in successive generations, sometimes quite dramatically. Occasionally, decreases in the av-

verage repeat length are observed (Fu *et al.*, *Science* 255:1256-1259 (1992); Yu *et al.*, *Am. J. Hum. Genet.* 50:968-980 (1992); Bruner *et al.*, *N. Engl. J. Med.*:476-480 (1993)). The *HD* trinucleotide repeat is also unstable, usually expanding when transmitted to the next generation, but contracting on occasion. In *HD*, as in the other disorders, change in copy number occurs in the absence of recombination. Compared with the fragile X syndrome, myotonic dystrophy, and *HD*, the instability of the disease allele in spino-bulbar muscular atrophy is more limited, and dramatic expansions of repeat length have not been seen (Biancalana *et al.*, *Hum. Mol. Genet.* 1:255-258 (1992)).

5 [0126] Expansion of the repeat length in myotonic dystrophy is associated with a particular chromosomal haplotype, suggesting the existence of a primordial predisposing mutation (Harley *et al.*, *Am. J. Hum. Genet.* 49:68-75 (1991); Harley *et al.*, *Nature* 355:545-546 (1992); Ashizawa, *Lancet* 338:642-643 (1991); and Epstein (1991)). In the fragile X syndrome, there may be a limited number of ancestral mutations that predispose to increases in trinucleotide repeat number (Richards *et al.*, *Nature Genet.* 1:257-260 (1992); Oudet *et al.*, *Am. J. Hum. Genet.* 52:297-304 (1993)). The linkage disequilibrium analysis used to identify IT15 indicates that there are several haplotypes associated with *HD*, but that at least 1/3 of *HD* chromosomes are ancestrally related (MacDonald *et al.*, *Nature Genet.* 1:99-103 (1992)). These data, combined with the reported low rate of new mutation to *HD* (Harper, *J. Med. Genet.* 29:365-376 (1992)), suggest that expansion of the trinucleotide repeat may only occur on select chromosomes. The analysis of two families presented herein, in which new mutation was supposed to have occurred, is consistent with the view that there may be particular normal chromosomes that have the capacity to undergo expansion of the repeat into the *HD* range. In each of these families, a chromosome with a (CAG)<sub>n</sub> repeat length in the upper end of the normal range was segregating on a chromosome whose 4p16.3 haplotype matched the most common haplotype seen on *HD* chromosomes and the clinical appearance of *HD* in these two cases was associated with expansion of the trinucleotide repeat.

10 [0127] The recent application of haplotype analysis to explore the linkage disequilibrium on *HD* chromosomes pointed to a portion of a 2.2 Mb candidate region defined by the majority of recombination events described in *HD* pedigrees (MacDonald *et al.*, *Nature Genet.* 1:99-103 (1992)). Previously, the search for the gene was confounded by three matings in which the genetic inheritance pattern was inconsistent with the remainder of the family (MacDonald *et al.*, *Neuron* 3:183-190 (1989b); Prichard *et al.*, *Am. J. Hum. Genet.* 50:1218-1230 (1992)). These matings produced apparently affected *HD* individuals despite the inheritance of only normal alleles for markers throughout 4p16.3, effectively excluding inheritance of the *HD* chromosome present in the rest of the pedigree. Using PCR assay disclosed above, each of these families was tested and it was determined that like other *HD* kindreds, an expanded allele segregates with *HD* in affected individuals of all three pedigrees. However, an expanded allele was not present in those specific individuals with the inconsistent 4p16.3 genotypes. Instead, these individuals displayed the normal alleles expected based on analysis of other markers in 4p16.3. It is conceivable that these inconsistent individuals do not, in fact, have *HD*, but some other disorder. Alternatively, they might represent genetic mosaics in which the *HD* allele is more heavily represented and/or more expanded in brain tissue than in the lymphoblast DNA used for genotyping.

15 [0128] The capacity to monitor directly the size of the trinucleotide repeat in individuals "at risk" for *HD* provides significant advantages over current methods, eliminating the need for complicated linkage analyses, facilitating genetic counseling, and extending the applicability of presymptomatic and prenatal diagnosis to "at risk" individuals with no living affected relatives. However, it is of the utmost importance that the current internationally accepted guidelines and counseling protocols for testing those "at risk" continue to be observed, and that samples from unaffected relatives should not be tested inadvertently or without full consent. In the series of patients examined in this study, there is an apparent correlation between repeat length and age of onset of the disease, reminiscent of that reported in myotonic dystrophy (Harley *et al.*, *Lancet* 339:1125-1128 (1992); Tsilfidis *et al.*, *Nature Genet.* 1:192-195 (1992)). The largest *HD* trinucleotide repeat segments were found in juvenile onset cases, where there is a known preponderance of male transmission (Merrit *et al.*, *Excerpta Medica*, Amsterdam, pp. 645-650 (1969)).

20 [0129] The expression of fragile X syndrome is associated with direct inactivation of the *FMR1* gene (Pieretti *et al.*, *Cell* 66:817-822 (1991); DeBoule *et al.*, *Nature Genet.* 3:31-35 (1993)). The recessive inheritance pattern of spinobulbar muscular atrophy suggests that in this disorder, an inactive gene product is produced. In myotonic dystrophy, the manner in which repeat expansion leads to the dominant disease phenotype is unknown. There are numerous possibilities for the mechanism of pathogenesis of the expanded trinucleotide repeat in *HD*. Without intending to be held to this theory, nevertheless notice can be taken that since Wolf-Hirschhorn patients hemizygous for 4p16.3 do not display features of *HD*, and IT15 mRNA is present in *HD* homozygotes, the expanded trinucleotide repeat does not cause simple inactivation of the gene containing it. The observation that the phenotype of *HD* is completely dominant, since homozygotes for the disease allele do not differ clinically from heterozygotes, has suggested that *HD* results from a gain of function mutation, in which either the mRNA product or the protein product of the disease allele would have some new property, or be expressed inappropriately (Wexler *et al.*, *Nature* 326:194-197 (1987); Myers *et al.*, *Am. J. Hum. Genet.* 45:615-618 (1989)). If the expanded trinucleotide repeat were translated, the consequences on the protein product would be dramatic, increasing the length of the poly-glutamine stretch near the N-terminus. It is possible, however, that despite the presence of an upstream Met codon, the normal translational start occurs 3' to the (CAG)<sub>n</sub> repeat and there is no poly-glutamine stretch in the protein product. In this case, the repeat would be in the 5' untrans-

lated region and might be expected to have its dominant effect at the mRNA level. The presence of an expanded repeat might directly alter regulation, localization, stability or translatability of the mRNA containing it, and could indirectly affect its counterpart from the normal allele in *HD* heterozygotes. Other conceivable scenarios are that the presence of an expanded repeat might alter the effective translation start site for the *HD* transcript, thereby truncating the protein, or alter the transcription start site for the *IT15* gene, disrupting control of mRNA expression. Finally, although the repeat is located within the *IT15* transcript, the possibility that it leads to *HD* by virtue of an action on the expression of an adjacent gene cannot be excluded.

[0130] Despite this final caveat, it is consistent with the above results and most likely that the trinucleotide repeat expansion causes *HD* by its effect, either at the mRNA or protein level, on the expression and/or structure of the protein product of the *IT15* gene, which has been named huntingtin. Outside of the region of the triplet repeat, the *IT15* DNA sequence detected no significant similarity to any previously reported gene in the GenBank database. Except for the stretches of glutamine and proline near the N-terminus, the amino acid sequence displayed no similarity to known proteins, providing no conspicuous clues to huntingtin's function. The poly-glutamine and poly-proline region near the N-terminus detect similarity with a large number of proteins which also contain long stretches of these amino acids. It is difficult to assess the significance of such similarities, although it is notable that many of these are DNA binding proteins and that huntingtin does have a single leucine zipper motif at residue 1,443. Huntingtin appears to be widely expressed, and yet cell death in *HD* is confined to specific neurons in particular regions of the brain.

TABLE 1.

COMPARISON OF HD AND NORMAL REPEAT SIZES				
RANGE OF ALLELE SIZES (#REPEATS)	NORMAL CHROMOSOMES NUMBER AND FREQUENCY		HD CHROMOSOMES NUMBER AND FREQUENCY	
≥ 48	0	0	44	0.59
42-47	0	0	30	0.41
30-41	2	0.01	0	0
25-30	2	0.01	0	0
≤ 24	169	0.98	0	0
TOTAL	173	1.00	74	1.0

### Example 5

#### *Distribution of Trinucleotide Repeat Lengths on Normal and HD Chromosomes*

[0131] The number of copies of the *HD* triplet repeat has been examined in a total of 425 *HD* chromosomes from 150 independent families and compared with the copy number of the *HD* triplet repeat of 545 normal chromosomes. The results are displayed in Figure 11. Two non-overlapping distributions of repeat length were observed, wherein the upper end of the normal range and the lower end of the *HD* range were separated by 3 repeat units. The normal chromosomes displayed 24 alleles producing PCR products ranging from 11 to 34 repeat units, with a median of 19 units (mean 19.71, s.d. 3.21). The *HD* chromosomes yielded 54 discrete PCR products corresponding to repeat lengths of 37 to 86 units, with a median of 45 units (mean 46.42, s.d. 6.68).

[0132] Of the *HD* chromosomes, 134 and 161 were known to be maternally or paternally-derived, respectively. To investigate whether the sex of the transmitting parent might influence the distribution of repeat lengths, these two sets of chromosomes were plotted separately in Figure 12. The maternally-derived chromosomes displayed repeat lengths ranging from 37 to 73 units, with a median of 44 (mean 44.93, s.d. 5.14). The paternally-derived chromosomes had 37 to 86 copies of the repeat unit, with a median of 48 units (mean 49.14, s.d. 8.27). However, a higher proportion of the paternally-derived *HD* chromosomes had repeat lengths greater than 55 units (16% vs. 2%), suggesting the possibility of a differential effect of paternal versus maternal transmission.

[0133] The data set used excluded chromosomes from a few clinically diagnosed individuals who have previously been shown not to have inherited the *HD* chromosome by DNA marker linkage studies (MacDonald, M.E., *et al.*, *Neuron* 3:183-190 (1989); Pritchard, C., *et al.*, *Am. J. Hum. Genet.* 50:1218-1230 (1992)). These individuals have repeat lengths well within the normal range. Their disease manifestations have not been explained, and they may represent phenocopies of *HD*. Regardless of the mechanism involved, the occurrence at low frequency of such individuals within known *HD* families must be considered if diagnostic conclusions are based solely on repeat length.

[0134] The control data set also excludes a number of chromosomes from phenotypically normal individuals who are related to "spontaneous" cases of HD or "new mutations". Chromosomes from these individuals who are not clinically affected and have no family history of the disorder cannot be designated as HD. However, these chromosomes cannot be classified as unambiguously normal because they are essentially the same chromosome as that of an affected relative, the diagnosed "spontaneous" HD proband, except with respect to repeat length. The lengths of repeat found on these ambiguous chromosomes (34-38 units) span the gap between the control and HD distributions, confounding a decision on the status of any individual with a repeat in the high normal to low HD range.

**Example 6**

*Instability of the Trinucleotide Repeat*

[0135] The data in Figure 11 combine repeat lengths from 150 different HD families representing many potentially independent origins of the defect. To examine the variation in repeat lengths on sets of HD chromosomes known to descend from a common founder, the data from three large HD kindreds (Gusella, J.F., *et al.*, *Nature* 306:234-238 (1983); Wexler, N.S., *et al.*, *Nature* 326:194-197 (1987); Folstein, S.E., *et al.*, *Science* 229:776-779 (1985)) with different 4p16.3 haplotypes (MacDonald, M.E., *et al.*, *Nature Genet.* 1:99-103 (1992)), typed for 75, 25 and 35 individuals, respectively, were separated. Despite the single origin of the founder HD chromosome within each pedigree, members of the separate pedigrees display a wide range of repeat lengths (Figure 13). This instability of the HD chromosome repeat is most prominent in members of a large Venezuelan HD kindred (panel A) in which the common HD ancestor has produced 10 generations of descendants, numbering over 13,000 individuals. The distribution of repeat lengths in this sampling of the Venezuelan pedigree (median 46, mean 48.26, s.d. 9.3) is not significantly different from that of the larger sample of HD chromosomes from all families. Panels B and C display results for two extended families in which HD was introduced more recently than in the Venezuelan kindred. These families have been reported to exhibit different age of onset distributions and varied phenotypic features of HD (Folstein, S.E., *et al.*, *Science* 229:776-779 (1985)). Both revealed extensive repeat length variation, with a median of 41 and 49 repeat units, respectively. The distribution of repeat lengths in the members of the family in Panel B was significantly different from the distribution of all HD chromosome repeat lengths ( $p<0.0001$ ), with a smaller mean of 42.04 repeat units (s.d. 2.82). The repeat distribution from HD chromosomes of Panel C was also significantly different from the total data set ( $p<0.004$ ), but with a higher mean of 49.80 (s.d. 5.86).

**Example 7**

*Parental Source Effects on Repeat Length Variation*

[0136] For 62 HD chromosomes in Figure 11, the length of the trinucleotide repeat also could be examined on the corresponding parental HD chromosome. In 20 of 25 maternal transmissions, and in 31 of 37 paternal transmissions, the repeat length was altered, indicating considerable instability. A similar phenomenon was not observed for normal chromosomes, where more than 500 meiotic transmissions revealed no changes in repeat length, although the very existence of such a large number of normal alleles suggests at least a low degree of instability.

[0137] Figure 14 shows the relationship between the repeat lengths on the HD chromosomes in the affected parent and corresponding progeny. For the 20 maternally-inherited chromosomes on which the repeat length was altered, 13 changes were increases in length and 7 were decreases. Both increases and decreases involved changes of less than 5 repeat units and the overall correlation between the mother's repeat length and that of her child was  $r=0.95$  ( $p<0.0001$ ).

[0138] The average change in repeat length in the 25 maternal transmissions was an increase of 0.4 repeats.

[0139] On paternally-derived chromosomes, the 31 transmissions in which the repeat length changes comprised 26 length increases and 5 length decreases. Although the decreases in size were only slightly smaller than those observed on maternally-derived chromosomes, ranging from 1 to 3 repeat units, the increases were often dramatically larger. Thus, the correlation of the repeat length in the father with that of his offspring was only  $r=0.35$  ( $p<0.04$ ). The average change in the 37 paternal transmissions was an increase of 9 repeat units. The maximum length increase observed through paternal transmission was 41 repeat units, a near doubling of the parental repeat.

[0140] For both male and female transmissions, there was no correlation between the size of the parental repeat and either the magnitude or frequency of changes.

[0141] To determine whether the variation in the length of the repeat observed through male transmission of HD chromosomes is reflected in the male germ cells, we amplified the repeat from sperm DNA and from DNA of the corresponding lymphoblast from 5 HD gene carriers. The results, shown in Figure 15, reveal striking differences between the lymphoblast and sperm DNA for the HD chromosome repeat, but not for the repeat on the normal chromosome. All the sperm donors are members of the Venezuelan HD family and range in age from 24 to 30 years. Individuals

1 and 2 are siblings with HD chromosome repeat lengths based on lymphoblast DNA of 45 and 52, respectively. Individuals 3 and 4 are also siblings, with HD repeat lengths of 46 and 49, respectively. Individual 5, from a different sibship than either of the other two pairs, has an HD repeat of 52 copies. In all 5 cases, the PCR amplification of sperm DNA and lymphoblast DNA yielded identical products from the normal chromosome. However, in comparison with lymphoblast DNA, the HD gene from sperm DNA yielded a diffuse array of products. In 3 of the 5 cases (2,4 and 5), the diffuse array spread to much larger allelic products than the corresponding lymphoblast product. Subject 2 showed the greatest range of expansion, with the sperm DNA product extending to over 80 repeat units. Interestingly, the 3 individuals displaying the greatest variation have the longest repeats and are currently symptomatic. The other two donors have shorter repeat lengths in the HD range, and remain at risk at this time.

5 [0141] The striking difference in the high repeat length range (>55) between HD chromosomes transmitted from the father and those transmitted from the mother indicated a potential parental source effect. When this was examined directly, the HD chromosome repeat length changed in about 85% of transmissions. Most changes involved a fluctuation of only a few repeat units, with larger increases occurring only in male transmissions. The greater size increases in male transmission appear to be caused by particular instability of the HD trinucleotide repeat during male gametogenesis, based on the amplification of the repeat from sperm DNA.

10 **Example 8**

20 *Relationship between Repeat Length and Age of Onset*

25 [0142] Increased repeat length might correlate with a reduced age of onset of HD. Accordingly, age of onset data was determined for 234 of the individuals represented in Figure 11. Figure 16 displays the repeat lengths found on the HD and normal chromosomes of these individuals relative to their age of onset. Indeed, age of onset is inversely correlated with the HD repeat length. A Pearson correlation coefficient of  $r=-.75$ ,  $p <0.0001$  was obtained assuming a linear relationship between age of onset and repeat length. When a polynomial function was used, a better fit was obtained ( $R^2=0.61$ ,  $F=121.45$ ), suggesting a higher order association between age of onset and repeat length.

30 [0143] There is considerable variation in the age of onset associated with any specific number of repeat units, particularly for trinucleotide repeats in the 37-52 unit zone (88% of HD chromosomes) where onset ranged from 15 to 75 years. In this range, a linear relationship between age of onset and repeat length provided as good a fit as a higher order relationship. The 95% confidence interval surrounding the predicted regression line was estimated at  $\pm 18$  years. In the 37 to 52 unit range, the association of repeat length to onset age is only half as strong as in the overall distribution ( $r=-0.40$ ,  $p <.0001$ ), indicating that much of the predictive power is contributed by repeats longer than 52 units. In this increased range, onset is likely to be very young and consequently not relevant to most persons seeking testing.

35 [0144] For the 178 cases in the 37-52 repeat unit range for which it was possible to subdivide the data set based on parental origin of the HD gene, multivariate regression analysis suggested a significant effect of parental origin on age of onset ( $p < 0.05$ ) independent of repeat length in this range. HD gene carriers from maternal transmissions had an average age of onset two years later than those from paternal transmissions.

40 [0145] In both univariate and multivariate analyses, no association between age of onset and the repeat length on the normal chromosome was detected, either in the total data set, or when it was subdivided into chromosomes of maternal or paternal origin.

SEQUENCE LISTING

45 (1) GENERAL INFORMATION:

[0146]

(i) APPLICANT: THE GENERAL HOSPITAL CORPORATION

50 Fruit Street  
Boston, Massachusetts 02114  
United States of America

(ii) TITLE OF INVENTION: Huntingtin DNA, Protein And Uses Thereof

55 (iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:

5 (A) KILBURN & STRODE  
(B) 30 JOHN STREET  
(C) LONDON  
(D) GREAT BRITAIN  
(E) WC1N 2DD

(v) COMPUTER READABLE FORM:

10 (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: Patentin Release #1.0, Version #1.25

15 (vi) CURRENT APPLICATION DATA:

(A) 7th March 1994

(vii) PRIOR APPLICATION DATA:

20 (A) APPLICATION NUMBER: 08/085,000  
(B) FILING DATE: 01 JULY 1993

(viii) PRIOR APPLICATION DATA:

25 (A) APPLICATION NUMBER: 08/027,498  
(B) FILING DATE: 05 MARCH 1993

(2) INFORMATION FOR SEQ ID NO:1:

30 [0147]

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCGGGAGAC CGCCATGGCG

20

(2) INFORMATION FOR SEQ ID NO:2:

45 [0148]

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 17 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AATACGACTC ACTATAG

17

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- 5 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

10 ATGAAGGCCT TCGAGTCCCT CAAGTCCTTC

30

(2) INFORMATION FOR SEQ ID NO:4:

15 [0149]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- 20 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

25 AAACTCACGG TCGGTGCAGC GGCTCCTCAG

30

(2) INFORMATION FOR SEQ ID NO:5:

30 [0150]

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 10366 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 316..9748

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

50

55

## EP 0 614 977 B1

	TTGCTGTGTG AGGCAGAACCG TGCAGGGGCA GGGGCGGGCT GGTTCCCTGG CCAGCCATTG	60
5	GCAGAGTCCG CAGGCTAGGG CTGTCAATCA TGCTGGCCGG CGTGGCCCCG CCTCCGCCGG	120
	CGCGGCCCG CCTCCGCCGG CGCACGTCTG GGACGCAAGG CGCCGTGGGG GCTGCCGGGA	180
	CGGGTCCAAG ATGGACGGCC GCTCAGGTT TGCTTTTACC TGCGGCCAG AGCCCCATTC	240
	ATTGCCCGG TGCTGAGCGG CGCCGCGAGT CGGCCGAGG CCTCCGGGA CTGCCGTGCC	300
10	GGGCGGGAGA CCGCC ATG GCG ACC CTG GAA AAG CTG ATG AAG GCC TTC GAG Met Ala Thr Leu Glu Lys Leu Met Lys Ala Phe Glu	351
	1 5 10	
15	TCC CTC AAG TCC TTC CAG CAG Ser Leu Lys Ser Phe Gln	399
	15 20 25	
	CAG CAG CAG CAG CAG CAG CAG CAG CAG CAA CAG CCG CCA CCG CCG Gln Gln Pro Pro Pro Pro	447
	30 35 40	
20	CCG CCG CCG CCG CCT CCT CAG CTT CCT CAG CCG CCG CCG CAG GCA Pro Pro Pro Pro Pro Pro Gln Leu Pro Gln Pro Pro Pro Gln Ala	495
	45 50 55 60	
	CAG CCG CTG CTG CCT CAG CCG CAG CCG CCC CCG CCG CCG CCC CCG CCG Gln Pro Leu Leu Pro Gln Pro Pro Pro Pro Pro Pro Pro Pro Pro	543
	65 70 75	
25	CCA CCC GGC CCG GCT GTG GCT GAG GAG CCG CTG CAC CGA CCA AAG AAA	591

30

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	Pro Pro Gly Pro Ala Val Ala Glu Glu Pro Leu His Arg Pro Lys Lys	
	80 85 90	
5	GAA CTT TCA GCT ACC AAG AAA GAC CGT GTG AAT CAT TGT CTG ACA ATA Glu Leu Ser Ala Thr Lys Lys Asp Arg Val Asn His Cys Leu Thr Ile 95 100 105	639
	TGT GAA AAC ATA GTG GCA CAG TCT GTC AGA AAT TCT CCA GAA TTT CAG Cys Glu Asn Ile Val Ala Gln Ser Val Arg Asn Ser Pro Glu Phe Gln 110 115 120	687
10	AAA CTT CTG GGC ATC GCT ATG GAA CTT TTT CTG CTG TGC AGT GAT GAC Lys Leu Leu Gly Ile Ala Met Glu Leu Phe Leu Leu Cys Ser Asp Asp 125 130 135 140	735
15	GCA GAG TCA GAT GTC AGG ATG GTG GCT GAC GAA TGC CTC AAC AAA GTT Ala Glu Ser Asp Val Arg Met Val Ala Asp Glu Cys Leu Asn Lys Val 145 150 155	783
	ATC AAA GCT TTG ATG GAT TCT AAT CTT CCA AGG TTA CAG CTC GAG CTC Ile Lys Ala Leu Met Asp Ser Asn Leu Pro Arg Leu Gln Leu Glu Leu 160 165 170	831
20	TAT AAG GAA ATT AAA AAG AAT GGT GCC CCT CGG AGT TTG CGT GCT GCC Tyr Lys Glu Ile Lys Lys Asn Gly Ala Pro Arg Ser Leu Arg Ala Ala 175 180 185	879
	CTG TGG AGG TTT GCT GAG CTG GCT CAC CTG GTT CGG CCT CAG AAA TGC Leu Trp Arg Phe Ala Glu Leu Ala His Leu Val Arg Pro Gln Lys Cys 190 195 200	927
25	AGG CCT TAC CTG GTG AAC CTT CTG CCG TGC CTG ACT CGA ACA AGC AAG Arg Pro Tyr Leu Val Asn Leu Leu Pro Cys Leu Thr Arg Thr Ser Lys 205 210 215 220	975
	AGA CCC GAA GAA TCA GTC CAG GAG ACC TTG GCT GCA GCT GTT CCC AAA Arg Pro Glu Glu Ser Val Gln Glu Thr Leu Ala Ala Val Pro Lys 225 230 235	1023
30	ATT ATG GCT TCT TTT GGC AAT TTT GCA AAT GAC AAT GAA ATT AAG GTT Ile Met Ala Ser Phe Gly Asn Phe Ala Asn Asp Asn Glu Ile Lys Val 240 245 250	1071
	TTG TTA AAG GCC TTC ATA GCG AAC CTG AAG TCA AGC TCC CCC ACC ATT Leu Leu Lys Ala Phe Ile Ala Asn Leu Lys Ser Ser Pro Thr Ile 255 260 265	1119
35	CGG CGG ACA GCG GCT GGA TCA GCA GTG AGC ATC TGC CAG CAC TCA AGA Arg Arg Thr Ala Ala Gly Ser Ala Val Ser Ile Cys Gln His Ser Arg 270 275 280	1167
	AGG ACA CAA TAT TTC TAT AGT TGG CTA CTA AAT GTG CTC TTA GGC TTA Arg Thr Gln Tyr Phe Tyr Ser Trp Leu Leu Asn Val Leu Leu Gly Leu 285 290 295 300	1215
40	CTC GTT CCT GTC GAG GAT GAA CAC TCC ACT CTG CTG ATT CTT GGC GTG Leu Val Pro Val Glu Asp Glu His Ser Thr Leu Leu Ile Leu Gly Val 305 310 315	1263
	CTG CTC ACC CTG AGG TAT TTG GTG CCC TTG CTG CAG CAG CAG GTC AAG Leu Leu Thr Leu Arg Tyr Leu Val Pro Leu Leu Gln Gln Val Lys 320 325 330	1311
45	GAC ACA AGC CTG AAA GGC AGC TTC GGA GTG ACA AGG AAA GAA ATG GAA Asp Thr Ser Leu Lys Gly Ser Phe Gly Val Thr Arg Lys Glu Met Glu 335 340 345	1359
	GTC TCT CCT TCT GCA GAG CAG CTT GTC CAG GTT TAT GAA CTG ACG TTA Val Ser Pro Ser Ala Glu Gln Leu Val Gln Val Tyr Glu Leu Thr Leu 350 355 360	1407
50	CAT CAT ACA CAG CAC CAA GAC CAC AAT GTT GTG ACC GGA GCC CTG GAG His His Thr Gln His Gln Asp His Asn Val Val Thr Gly Ala Leu Glu	1455

	365	370	375	380	
5	CTG TTG CAG CAG CTC TTC AGA ACG CCT CCA CCC GAG CTT CTG CAA ACC Leu Leu Gln Gln Leu Phe Arg Thr Pro Pro Pro Glu Leu Leu Gln Thr 385 390 395				1503
10	CTG ACC GCA GTC GGG GGC ATT GGG CAG CTC ACC GCT GCT AAG GAG GAG Leu Thr Ala Val Gly Gly Ile Gly Gln Leu Thr Ala Ala Lys Glu Glu 400 405 410				1551
15	TCT GGT GGC CGA AGC CGT AGT GGG AGT ATT GTG GAA CTT ATA GCT GGA Ser Gly Gly Arg Ser Arg Ser Gly Ser Ile Val Glu Leu Ile Ala Gly 415 420 425				1599
20	GGG GGT TCC TCA TGC AGC CCT GTC CTT TCA AGA AAA CAA AAA GCC AAA Gly Gly Ser Ser Cys Ser Pro Val Leu Ser Arg Lys Gln Lys Gly Lys 430 435 440				1647
25	GTG CTC TTA GGA GAA GAA GCC TTG GAG GAT GAC TCT GAA TCG AGA Val Leu Leu Gly Glu Ala Leu Glu Asp Asp Ser Glu Ser Arg 445 450 455 460				1695
30	TCG GAT GTC AGC AGC TCT GCC TTA ACA GCC TCA GTG AAG GAT GAG ATC Ser Asp Val Ser Ser Ala Leu Thr Ala Ser Val Lys Asp Glu Ile 465 470 475				1743
35	AGT GGA GAG CTG GCT GCT TCT TCA GGG GTT TCC ACT CCA GGG TCA GCA Ser Gly Glu Leu Ala Ala Ser Ser Gly Val Ser Thr Pro Gly Ser Ala 480 485 490				1791
40	GGT CAT GAC ATC ATC ACA GAA CAG CCA CGG TCA CAG CAC ACA CTG CAG Gly His Asp Ile Ile Thr Glu Gln Pro Arg Ser Gln His Thr Leu Gln 495 500 505				1839
45	GCG GAC TCA CTG GAT CTG GCC AGC TGT GAC TTG ACA AGC TCT GCC ACT Ala Asp Ser Leu Asp Leu Ala Ser Cys Asp Leu Thr Ser Ser Ala Thr 510 515 520				1887
50	GAT GGG GAT GAG GAG GAT ATC TTG AGC CAC AGC TCC AGC CAG GTC AGC Asp Gly Asp Glu Glu Asp Ile Leu Ser His Ser Ser Gln Val Ser 525 530 535 540				1935
55	GCC GTC CCA TCT GAC CCT GCC ATG GAC CTG AAT GAT GGG ACC CAG GCC Ala Val Pro Ser Asp Pro Ala Met Asp Leu Asn Asp Gly Thr Gln Ala 545 550 555				1983
60	TCG TCG CCC ATC AGC GAC AGC TCC CAG ACC ACC ACC GAA GGG CCT GAT Ser Ser Pro Ile Ser Asp Ser Ser Gln Thr Thr Glu Gly Pro Asp 560 565 570				2031
65	TCA GCT GTT ACC CCT TCA GAC AGT TCT GAA ATT GTG TTA GAC GGT ACC Ser Ala Val Thr Pro Ser Asp Ser Ser Glu Ile Val Leu Asp Gly Thr 575 580 585				2079
70	GAC AAC CAG TAT TTG GGC CTG CAG ATT GGA CAG CCC CAG GAT GAA GAT Asp Asn Gln Tyr Leu Gly Leu Gln Ile Gly Gln Pro Gln Asp Glu Asp 590 595 600				2127
75	GAG GAA GCC ACA GGT ATT CTT CCT GAT GAA GCC TCG GAG GCC TTC AGG Glu Glu Ala Thr Gly Ile Leu Pro Asp Glu Ala Ser Glu Ala Phe Arg 605 610 615 620				2175
80	AAC TCT TCC ATG GCC CTT CAA CAG GCA CAT TTA TTG AAA AAC ATG AGT Asn Ser Ser Met Ala Leu Gln Gln Ala His Leu Leu Lys Asn Met Ser 625 630 635				2223
85	CAC TGC AGG CAG CCT TCT GAC AGC AGT GTT GAT AAA TTT GTG TTG AGA His Cys Arg Gln Pro Ser Asp Ser Ser Val Asp Lys Phe Val Leu Arg 640 645 650				2271
90	GAT GAA GCT ACT GAA CCG GGT GAT CAA GAA AAC AAG CCT TGC CGC ATC Asp Glu Ala Thr Glu Pro Gly Asp Gln Glu Asn Lys Pro Cys Arg Ile				2319

	655	660	665	
5	AAA GGT GAC ATT GGA CAG TCC ACT GAT GAT GAC TCT GCA CCT CTT GTC Lys Gly Asp Ile Gly Gln Ser Thr Asp Asp Asp Ser Ala Pro Leu Val 670 675 680			2367
10	CAT TCT GTC CGC CTT TTA TCT GCT TCG TTT TTG CTA ACA GGG GGA AAA His Ser Val Arg Leu Leu Ser Ala Ser Phe Leu Leu Thr Gly Gly Lys 685 690 695 700			2415
15	AAT GTG CTG GTT CCG GAC AGG GAT GTG AGG GTC AGC GTG AAG GCC CTG Asn Val Leu Val Pro Asp Arg Asp Val Arg Val Ser Val Lys Ala Leu 705 710 715			2463
20	GCC CTC AGC TGT GTG GGA GCA GCT GTG GCC CTC CAC CCG GAA TCT TTC Ala Leu Ser Cys Val Gly Ala Ala Val Ala Leu His Pro Glu Ser Phe 720 725 730 735			2511
25	TTC AGC AAA CTC TAT AAA GTT CCT CTT GAC ACC ACG GAA TAC CCT GAG Phe Ser Lys Leu Tyr Lys Val Pro Leu Asp Thr Thr Glu Tyr Pro Glu 735 740 745			2559
30	GAA CAG TAT GTC TCA GAC ATC TTG AAC TAC ATC GAT CAT GGA GAC CCA Glu Gln Tyr Val Ser Asp Ile Leu Asn Tyr Ile Asp His Gly Asp Pro 750 755 760			2607
35	CAG GTT CGA GGA GCC ACT GCC ATT CTC TGT GGG ACC CTC ATC TGC TCC Gln Val Arg Gly Ala Thr Ala Ile Leu Cys Gly Thr Leu Ile Cys Ser 765 770 775 780			2655
40	ATC CTC AGC AGG TCC CGC TTC CAC GTG GGA GAT TGG ATG GGC ACC ATT Ile Leu Ser Arg Ser Arg Phe His Val Gly Asp Trp Met Gly Thr Ile 785 790 795			2703
45	AGA ACC CTC ACA GGA AAT ACA TTT TCT TTG GCG GAT TGC ATT CCT TTG Arg Thr Leu Thr Gly Asn Thr Phe Ser Leu Ala Asp Cys Ile Pro Leu 800 805 810			2751
50	CTG CGG AAA ACA CTG AAG GAT GAG TCT TCT GTT ACT TGC AAG TTA GCT Leu Arg Lys Thr Leu Lys Asp Glu Ser Ser Val Thr Cys Lys Leu Ala 815 820 825			2799
55	TGT ACA GCT GTG AGG AAC TGT GTC ATG AGT CTC TGC AGC AGC AGC TAC Cys Thr Ala Val Arg Asn Cys Val Met Ser Leu Cys Ser Ser Ser Tyr 830 835 840			2847
	AGT GAG TTA GGA CTG CAG CTG ATC ATC GAT GTG CTG ACT CTG AGG AAC Ser Glu Leu Gly Leu Gln Leu Ile Ile Asp Val Leu Thr Leu Arg Asn 845 850 855 860			2895
	AGT TCC TAT TGG CTG GTG AGG ACA GAG CTT CTG GAA ACC CTT GCA GAG Ser Ser Tyr Trp Leu Val Arg Thr Glu Leu Leu Glu Thr Leu Ala Glu 865 870 875			2943
	ATT GAC TTC AGG CTG GTG AGC TTT TTG GAG GCA AAA GCA GAA AAC TTA Ile Asp Phe Arg Leu Val Ser Phe Leu Glu Ala Lys Ala Glu Asn Leu 880 885 890			2991
	CAC AGA GGG GCT CAT CAT TAT ACA GGG CTT TTA AAA CTG CAA GAA CGA His Arg Gly Ala His His Tyr Thr Gly Leu Leu Lys Leu Gln Glu Arg 895 900 905			3039
	GTC CTC AAT AAT GTT GTC ATC CAT TTG CTT GGA GAT GAA GAC CCC AGG Val Leu Asn Asn Val Val Ile His Leu Leu Gly Asp Glu Asp Pro Arg 910 915 920			3087
	GTC CGA CAT GTT GCC GCA GCA TCA ATT AGG CTT GTC CCA AAG CTG Val Arg His Val Ala Ala Ser Leu Ile Arg Leu Val Pro Lys Leu 925 930 935 940			3135
	TTT TAT AAA TGT GAC CAA GGA CAA GCT GAT CCA GTC GTG GCC GTG GCA Phe Tyr Lys Cys Asp Gln Gly Gln Ala Asp Pro Val Val Ala Val Ala			3183

	945	950	955	
5	AGA GAT CAA AGC AGT GTT TAC CTG AAA CTT CTC ATG CAT GAG ACG CAG Arg Asp Gln Ser Ser Val Tyr Leu Lys Leu Leu Met His Glu Thr Gln 960 965 970			3231
10	CCT CCA TCT CAT TTC TCC GTC AGC ACA ATA ACC AGA ATA TAT AGA GGC Pro Pro Ser His Phe Ser Val Ser Thr Ile Thr Arg Ile Tyr Arg Gly 975 980 985			3279
15	TAT AAC CTA CTA CCA AGC ATA ACA GAC GTC ACT ATG GAA AAT AAC CTT Tyr Asn Leu Leu Pro Ser Ile Thr Asp Val Thr Met Glu Asn Asn Leu 990 995 1000			3327
20	TCA AGA GTT ATT GCA GCA GTT TCT CAT GAA CTA ATC ACA TCA ACC ACC Ser Arg Val Ile Ala Ala Val Ser His Glu Leu Ile Thr Ser Thr Thr 1005 1010 1015 1020			3375
25	AGA GCA CTC ACA TTT GGA TGC TGT GAA GCT TTG TGT CTT CTT TCC ACT Arg Ala Leu Thr Phe Gly Cys Cys Glu Ala Leu Cys Leu Leu Ser Thr 1025 1030 1035			3423
30	GCC TTC CCA GTT TGC ATT TGG AGT TTA GGT TGG CAC TGT GGA GTG CCT Ala Phe Pro Val Cys Ile Trp Ser Leu Gly Trp His Cys Gly Val Pro 1040 1045 1050			3471
35	CCA CTG AGT GCC TCA GAT GAG TCT AGG AAG AGC TGT ACC GTT GGG ATG Pro Leu Ser Ala Ser Asp Glu Ser Arg Lys Ser Cys Thr Val Gly Met 1055 1060 1065			3519
40	GCC ACA ATG ATT CTG ACC CTG CTC TCG TCA GCT TGG TTC CCA TTG GAT Ala Thr Met Ile Leu Thr Leu Leu Ser Ser Ala Trp Phe Pro Leu Asp 1070 1075 1080			3567
45	CTC TCA GCC CAT CAA GAT GCT TTG ATT TTG GCC GGA AAC TTG CTT GCA Leu Ser Ala His Gln Asp Ala Leu Ile Leu Ala Gly Asn Leu Leu Ala 1085 1090 1095 1100			3615
50	GCC AGT GCT CCC AAA TCT CTG AGA AGT TCA TGG GCC TCT GAA GAA GAA Ala Ser Ala Pro Lys Ser Leu Arg Ser Ser Trp Ala Ser Glu Glu Glu 1105 1110 1115			3663
55	GCC AAC CCA GCA GCC ACC AAG CAA GAG GAG GTC TGG CCA GCC CTG GGG Ala Asn Pro Ala Ala Thr Lys Gln Glu Glu Val Trp Pro Ala Leu Gly 1120 1125 1130			3711
	GAC CGG GCC CTG GTG CCC ATG GTG GAG CAG CTC TTC TCT CAC CTG CTG Asp Arg Ala Leu Val Pro Met Val Glu Gln Leu Phe Ser His Leu Leu 1135 1140 1145			3759
	AAG GTG ATT AAC ATT TGT GCC CAC GTC CTG GAT GAC GTG GCT CCT GGA Lys Val Ile Asn Ile Cys Ala His Val Leu Asp Asp Val Ala Pro Gly 1150 1155 1160			3807
	CCC GCA ATA AAG GCA GCC TTG CCT TCT CTA ACA AAC CCC CCT TCT CTA Pro Ala Ile Lys Ala Ala Leu Pro Ser Leu Thr Asn Pro Pro Ser Leu 1165 1170 1175 1180			3855
	AGT CCC ATC CGA CGA AAG GGG AAG GAG AAA GAA CCA GGA GAA CAA GCA Ser Pro Ile Arg Arg Lys Gly Lys Glu Pro Gly Glu Gln Ala 1185 1190 1195			3903
	TCT GTA CCG TTG AGT CCC AAG AAA GGC AGT GAG GCC AGT GCA GCT TCT Ser Val Pro Leu Ser Pro Lys Lys Gly Ser Glu Ala Ser Ala Ala Ser 1200 1205 1210			3951
	AGA CAA TCT GAT ACC TCA GGT CCT GTT ACA ACA AGT AAA TCC TCA TCA Arg Gln Ser Asp Thr Ser Gly Pro Val Thr Thr Ser Lys Ser Ser Ser 1215 1220 1225			3999
	CTG GGG AGT TTC TAT CAT CTT CCT TCA TAC CTC AGA CTG CAT GAT GTC Leu Gly Ser Phe Tyr His Leu Pro Ser Tyr Leu Arg Leu His Asp Val			4047

	1230	1235	1240	
5	CTG AAA GCT ACA CAC GCT AAC TAC AAG GTC ACG CTG GAT CTT CAG AAC Leu Lys Ala Thr His Ala Asn Tyr Lys Val Thr Leu Asp Leu Gln Asn 1245 1250 1255 1260			4095
10	AGC ACG GAA AAG TTT GGA GGG TTT CTC CGC TCA GCC TTG GAT GTT CTT Ser Thr Glu Lys Phe Gly Gly Phe Leu Arg Ser Ala Leu Asp Val Leu 1265 1270 1275			4143
15	TCT CAG ATA CTA GAG CTG GCC ACA CTG CAG GAC ATT GGG AAG TGT GTT Ser Gln Ile Leu Glu Leu Ala Thr Leu Gln Asp Ile Gly Lys Cys Val 1280 1285 1290			4191
20	GAA GAG ATC CTA GGA TAC CTG AAA TCC TGC TTT AGT CGA GAA CCA ATG Glu Glu Ile Leu Gly Tyr Leu Lys Ser Cys Phe Ser Arg Glu Pro Met 1295 1300 1305			4239
25	ATG GCA ACT GTT TGT GTT CAA CAA TTG TTG AAG ACT CTC TTT GGC ACA Met Ala Thr Val Cys Val Gln Gln Leu Leu Lys Thr Leu Phe Gly Thr 1310 1315 1320			4287
30	AAC TTG GCC TCC CAG TTT GAT GGC TTA TCT TCC AAC CCC AGC AAG TCA Asn Leu Ala Ser Gln Phe Asp Gly Leu Ser Ser Asn Pro Ser Lys Ser 1325 1330 1335 1340			4335
35	CAA GGC CGA GCA CAG CGC CTT GGC TCC TCC AGT GTG AGG CCA GGC TTG Gln Gly Arg Ala Gln Arg Leu Gly Ser Ser Ser Val Arg Pro Gly Leu 1345 1350 1355			4383
40	TAC CAC TAC TGC TTC ATG GCC CCG TAC ACC CAC TTC ACC CAG GCC CTC Tyr His Tyr Cys Phe Met Ala Pro Tyr Thr His Phe Thr Gln Ala Leu 1360 1365 1370			4431
45	GCT GAC GCC AGC CTG AGG AAC ATG GTG CAG GCG GAG CAG GAG AAC GAC Ala Asp Ala Ser Leu Arg Asn Met Val Gln Ala Glu Gln Glu Asn Asp 1375 1380 1385			4479
50	ACC TCG GGA TGG TTT GAT GTC CTC CAG AAA GTG TCT ACC CAG TTG AAG Thr Ser Gly Trp Phe Asp Val Leu Gln Lys Val Ser Thr Gln Leu Lys 1390 1395 1400			4527
55	ACA AAC CTC ACG AGT GTC ACA AAG AAC CGT GCA GAT AAG AAT GCT ATT Thr Asn Leu Thr Ser Val Thr Lys Asn Arg Ala Asp Lys Asn Ala Ile 1405 1410 1415 1420			4575
60	CAT AAT CAC ATT CGT TTG TTT GAA CCT CTT GTT ATA AAA GCT TTA AAA His Asn His Ile Arg Leu Phe Glu Pro Leu Val Ile Lys Ala Leu Lys 1425 1430 1435			4623
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75	TCA GAT CAG GTG TTT ATT GGC TTT GTA TTG AAA CAG TTT GAA TAC ATT Ser Asp Gln Val Phe Ile Gly Phe Val Leu Lys Gln Phe Glu Tyr Ile 1470 1475 1480			4767
80	GAA GTG GGC CAG TTC AGG GAA TCA GAG GCA ATC ATT CCA AAC ATC TTT Glu Val Gly Gln Phe Arg Glu Ser Glu Ala Ile Ile Pro Asn Ile Phe 1485 1490 1495 1500			4815
85	TTC TTC TTG GTA TTA CTA TCT TAT GAA CGC TAT CAT TCA AAA CAG ATC Phe Phe Leu Val Leu Leu Ser Tyr Glu Arg Tyr His Ser Lys Gln Ile 1505 1510 1515			4863
90	ATT GGA ATT CCT AAA ATC ATT CAG CTC TGT GAT GGC ATC ATG GCC AGT Ile Gly Ile Pro Lys Ile Ile Gln Leu Cys Asp Gly Ile Met Ala Ser			4911

	1520	1525	1530	
5	GGA AGG AAG GCT GTG ACA CAT GCC ATA CCG GCT CTG CAG CCC ATA GTC Gly Arg Lys Ala Val Thr His Ala Ile Pro Ala Leu Gln Pro Ile Val 1535	1540	1545	4959
10	CAC GAC CTC TTT GTA TTA AGA GGA ACA AAT AAA GCT GAT GCA GGA AAA His Asp Leu Phe Val Leu Arg Gly Thr Asn Lys Ala Asp Ala Gly Lys 1550	1555	1560	5007
15	GAG CTT GAA ACC CAA AAA GAG GTG GTG GTG TCA ATG TTA CTG AGA CTC Glu Leu Glu Thr Gln Lys Glu Val Val Val Ser Met Leu Leu Arg Leu 1565	1570	1575	5055
20	ATC CAG TAC CAT CAG GTG TTG GAG ATG TTC ATT CTT GTC CTG CAG CAG Ile Gln Tyr His Gln Val Leu Glu Met Phe Ile Leu Val Leu Gln Gln 1585	1590	1595	5103
25	TGC CAC AAG GAG AAT GAA GAC AAG TGG AAG CGA CTG TCT CGA CAG ATA Cys His Lys Glu Asn Glu Asp Lys Trp Lys Arg Leu Ser Arg Gln Ile 1600	1605	1610	5151
30	GCT GAC ATC ATC CTC CCA ATG TTA GCC AAA CAG CAG ATG CAC ATT GAC Ala Asp Ile Ile Leu Pro Met Leu Ala Lys Gln Gln Met His Ile Asp 1615	1620	1625	5199
35	TCT CAT GAA GCC CTT GGA GTG TTA AAT ACA TTA TTT GAG ATT TTG GCC Ser His Glu Ala Leu Gly Val Leu Asn Thr Leu Phe Glu Ile Leu Ala 1630	1635	1640	5247
40	CCT TCC TCC CTC CGT CCG GTA GAC ATG CTT TTA CGG AGT ATG TTC GTC Pro Ser Ser Leu Arg Pro Val Asp Met Leu Leu Arg Ser Met Phe Val 1645	1650	1655	5295
45	ACT CCA AAC ACA ATG GCG TCC GTG AGC ACT GTT CAA CTG TGG ATA TCG Thr Pro Asn Thr Met Ala Ser Val Ser Thr Val Gln Leu Trp Ile Ser 1665	1670	1675	5343
50	GGA ATT CTG GCC ATT TTG AGG GTT CTG ATT TCC CAG TCA ACT GAA GAT Gly Ile Leu Ala Ile Leu Arg Val Leu Ile Ser Gln Ser Thr Glu Asp 1680	1685	1690	5391
55	ATT GTT CTT TCT CGT ATT CAG GAG CTC TCC TTC TCT CCG TAT TTA ATC Ile Val Leu Ser Arg Ile Gln Glu Leu Ser Phe Ser Pro Tyr Leu Ile 1695	1700	1705	5439
60	TCC TGT ACA GTA ATT AAT AGG TTA AGA GAT GGG GAC AGT ACT TCA ACG Ser Cys Thr Val Ile Asn Arg Leu Arg Asp Gly Asp Ser Thr Ser Thr 1710	1715	1720	5487
65	CTA GAA GAA CAC AGT GAA GGG AAA CAA ATA AAG AAT TTG CCA GAA GAA Leu Glu Glu His Ser Glu Gly Lys Gln Ile Lys Asn Leu Pro Glu Glu 1725	1730	1735	5535
70	ACA TTT TCA AGG TTT CTA TTA CAA CTG GTT GGT ATT CTT TTA GAA GAC Thr Phe Ser Arg Phe Leu Leu Gln Leu Val Gly Ile Leu Leu Glu Asp 1745	1750	1755	5583
75	ATT GTT ACA AAA CAG CTG AAG GTG GAA ATG AGT GAG CAG CAA CAT ACT Ile Val Thr Lys Gln Leu Lys Val Glu Met Ser Glu Gln Gln His Thr 1760	1765	1770	5631
80	TTC TAT TGC CAG GAA CTA GGC ACA CTG CTA ATG TGT CTG ATC CAC ATC Phe Tyr Cys Gln Glu Leu Gly Thr Leu Leu Met Cys Leu Ile His Ile 1775	1780	1785	5679
85	TTC AAG TCT GGA ATG TTC CGG AGA ATC ACA GCA GCT GCC ACT AGG CTG Phe Lys Ser Gly Met Phe Arg Arg Ile Thr Ala Ala Ala Thr Arg Leu 1790	1795	1800	5727
90	TTC CGC AGT GAT GGC TGT GGC AGT TTC TAC ACC CTG GAC AGC TTG Phe Arg Ser Asp Gly Cys Gly Ser Phe Tyr Thr Leu Asp Ser Leu			5775

	1805	1810	1815	1820	
5	AAC TTG CGG GCT CGT TCC ATG ATC ACC ACC CAC CCG GCC CTG GTG CTG Asn Leu Arg Ala Arg Ser Met Ile Thr Thr His Pro Ala Leu Val Leu 1825 1830 1835 5823				
10	CTC TGG TGT CAG ATA CTG CTG CTT GTC AAC CAC ACC GAC TAC CGC TGG Leu Trp Cys Gln Ile Leu Leu Leu Val Asn His Thr Asp Tyr Arg Trp 1840 1845 1850 5871				
15	TGG GCA GAA GTG CAG CAG ACC CCG AAA AGA CAC AGT CTG TCC AGC ACA Trp Ala Glu Val Gln Gln Thr Pro Lys Arg His Ser Leu Ser Ser Thr 1855 1860 1865 5919				
20	AAG TTA CTT AGT CCC CAG ATG TCT GGA GAA GAG GAT TCT GAC TTG Lys Leu Leu Ser Pro Gln Met Ser Gly Glu Glu Asp Ser Asp Leu 1870 1875 1880 5967				
25	GCA GCC AAA CTT GGA ATG TGC AAT AGA GAA ATA GTA CGA AGA GGG GCT Ala Ala Lys Leu Gly Met Cys Asn Arg Glu Ile Val Arg Arg Gly Ala 1885 1890 1895 6015				
30	CTC ATT CTC TTC TGT GAT TAT GTC TGT CAG AAC CTC CAT GAC TCC GAG Leu Ile Leu Phe Cys Asp Tyr Val Cys Gln Asn Leu His Asp Ser Glu 1905 1910 1915 6063				
35	CAC TTA ACG TGG CTC ATT GTA AAT CAC ATT CAA GAT CTG ATC AGC CTT His Leu Thr Trp Leu Ile Val Asn His Ile Gln Asp Leu Ile Ser Leu 1920 1925 1930 6111				
40	TCC CAC GAG CCT CCA GTA CAG GAC TTC ATC AGT GCC GTT CAT CGG AAC Ser His Glu Pro Pro Val Gln Asp Phe Ile Ser Ala Val His Arg Asn 1935 1940 1945 6159				
45	TCT GCT GCC AGC GGC CTG TTC ATC CAG GCA ATT CAG TCT CGT TGT GAA Ser Ala Ala Ser Gly Leu Phe Ile Gln Ala Ile Gln Ser Arg Cys Glu 1950 1955 1960 6207				
50	AAC CTT TCA ACT CCA ACC ATG CTG AAG AAA ACT CTT CAG TGC TTG GAG Asn Leu Ser Thr Pro Thr Met Leu Lys Lys Thr Leu Gln Cys Leu Glu 1965 1970 1975 1980 6255				
55	GGG ATC CAT CTC AGC CAG TCG GGA GCT GTG CTC ACG CTG TAT GTG GAC Gly Ile His Leu Ser Gln Ser Gly Ala Val Leu Thr Leu Tyr Val Asp 1985 1990 1995 6303				
60	AGG CTT CTG TGC ACC CCT TTC CGT GTG CTG GCT CGC ATG GTC GAC ATC Arg Leu Leu Cys Thr Pro Phe Arg Val Leu Ala Arg Met Val Asp Ile 2000 2005 2010 6351				
65	CTT GCT TGT CGC CGG GTA GAA ATG CTT CTG GCT GCA AAT TTA CAG AGC Leu Ala Cys Arg Arg Val Glu Met Leu Leu Ala Ala Asn Leu Gln Ser 2015 2020 2025 6399				
70	AGC ATG GCC CAG TTG CCA ATG GAA GAA CTC AAC AGA ATC CAG GAA TAC Ser Met Ala Gln Leu Pro Met Glu Glu Leu Asn Arg Ile Gln Glu Tyr 2030 2035 2040 6447				
75	CTT CAG AGC AGC GGG CTC GCT CAG AGA CAC CAA AGG CTC TAT TCC CTG Leu Gln Ser Ser Gly Leu Ala Gln Arg His Gln Arg Leu Tyr Ser Leu 2045 2050 2055 2060 6495				
80	CTG GAC AGG TTT CGT CTC TCC ACC ATG CAA GAC TCA CTT AGT CCC TCT Leu Asp Arg Phe Arg Leu Ser Thr Met Gln Asp Ser Leu Ser Pro Ser 2065 2070 2075 6543				
85	CCT CCA GTC TCT TCC CAC CCG CTG GAC GGG GAT GGG CAC GTG TCA CTG Pro Pro Val Ser Ser His Pro Leu Asp Gly Asp Gly His Val Ser Leu 2080 2085 2090 6591				
90	GAA ACA GTG AGT CCG GAC AAA GAC TGG TAC GTT CAT CTT GTC AAA TCC Glu Thr Val Ser Pro Asp Lys Asp Trp Tyr Val His Leu Val Lys Ser 2095 2100 2105 6639				

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	2095	2100	2105	
5	CAG TGT TGG ACC AGG TCA GAT TCT GCA CTG CTG GAA GGT GCA GAG CTG Gln Cys Trp Thr Arg Ser Asp Ser Ala Leu Leu Glu Gly Ala Glu Leu 2110 2115 2120			6687
10	GTG AAT CGG ATT CCT GCT GAA GAT ATG AAT GCC TTC ATG ATG AAC TCG Val Asn Arg Ile Pro Ala Glu Asp Met Asn Ala Phe Met Met Asn Ser 2125 2130 2135 2140			6735
15	GAG TTC AAC CTA AGC CTG CTA GCT CCA TGC TTA AGC CTA GGG ATG AGT Glu Phe Asn Leu Ser Leu Leu Ala Pro Cys Leu Ser Leu Gly Met Ser 2145 2150 2155			6783
20	GAA ATT TCT GGT GGC CAG AAG AGT GCC CTT TTT GAA GCA GCC CGT GAG Glu Ile Ser Gly Gly Gln Lys Ser Ala Leu Phe Glu Ala Ala Arg Glu 2160 2165 2170			6831
25	GTG ACT CTG GCC CGT GTG ACC GGC ACC GTG CAG CAG CTC CCT GCT GTC Val Thr Leu Ala Arg Val Ser Gly Thr Val Gln Gln Leu Pro Ala Val 2175 2180 2185			6879
30	CAT CAT GTC TTC CAG CCC GAG CTG CCT GCA GAG CCG GCG GCC TAC TGG His His Val Phe Gln Pro Glu Leu Pro Ala Glu Pro Ala Ala Tyr Trp 2190 2195 2200			6927
35	AGC AAG TTG AAT GAT CTG TTT GGG GAT GCT GCA CTG TAT CAG TCC CTG Ser Lys Leu Asn Asp Leu Phe Gly Asp Ala Ala Leu Tyr Gln Ser Leu 2205 2210 2215 2220			6975
40	CCC ACT CTG GCC CGG GCC CTG GCA CAG TAC CTG GTG GTG GTC TCC AAA Pro Thr Leu Ala Arg Ala Leu Ala Gln Tyr Leu Val Val Val Ser Lys 2225 2230 2235			7023
45	CTG CCC AGT CAT TTG CAC CTT CCT GAG AAA GAG AAG GAC ATT GTG Leu Pro Ser His Leu His Leu Pro Pro Glu Lys Glu Lys Asp Ile Val 2240 2245 2250			7071
50	AAA TTC GTG GTG GCA ACC CTT GAG GCC CTG TCC TGG CAT TTG ATC CAT Lys Phe Val Val Ala Thr Leu Glu Ala Leu Ser Trp His Leu Ile His 2255 2260 2265			7119
55	GAG CAG ATC CCG CTG AGT CTG GAT CTC CAG GCA GGG CTG GAC TGC TGC Glu Gln Ile Pro Leu Ser Leu Asp Leu Gln Ala Gly Leu Asp Cys Cys 2270 2275 2280			7167
60	TGC CTG GCC CTG CAG CTG CCT GGC CTC TGG AGC GTG GTC TCC TCC ACA Cys Leu Ala Leu Gln Leu Pro Gly Leu Trp Ser Val Val Ser Ser Thr 2285 2290 2295 2300			7215
65	GAG TTT GTG ACC CAC GCC TGC TCC CTC ATC TAC TGT GTG CAC TTC ATC Glu Phe Val Thr His Ala Cys Ser Leu Ile Tyr Cys Val His Phe Ile 2305 2310 2315			7263
70	CTG GAG GCC GTT GCA GTG CAG CCT GGA GAG CAG CTT CTT AGT CCA GAA Leu Glu Ala Val Ala Val Gln Pro Gly Glu Gln Leu Leu Ser Pro Glu 2320 2325 2330			7311
75	AGA AGG ACA AAT ACC CCA AAA GCC ATC AGC GAG GAG GAG GAA GTA Arg Arg Thr Asn Thr Pro Lys Ala Ile Ser Glu Glu Glu Glu Val 2335 2340 2345			7359
80	GAT CCA AAC ACA CAG AAT CCT AAG TAT ATC ACT GCA GCC TGT GAG ATG Asp Pro Asn Thr Gln Asn Pro Lys Tyr Ile Thr Ala Ala Cys Glu Met 2350 2355 2360			7407
85	GTG GCA GAA ATG GTG GAG TCT CTG CAG TCG GTG TTG GCC TTG GGT CAT Val Ala Glu Met Val Glu Ser Leu Gln Ser Val Leu Ala Leu Gly His 2365 2370 2375 2380			7455
90	AAA AGG AAT AGC GGC GTG CCG GCG TTT CTC ACG CCA TTG CTC AGG AAC Lys Arg Asn Ser Gly Val Pro Ala Phe Leu Thr Pro Leu Leu Arg Asn			7503

	2385	2390	2395	
5	ATC ATC ATC AGC CTG GCC CGC CTG CCC CTT GTC AAC AGC TAC ACA CGT Ile Ile Ile Ser Leu Ala Arg Leu Pro Leu Val Asn Ser Tyr Thr Arg 2400	2405	2410	7551
	GTG CCC CCA CTG GTG TGG AAG CTT GGA TGG TCA CCC AAA CCG GGA GGG Val Pro Pro Leu Val Trp Lys Leu Gly Trp Ser Pro Lys Pro Gly Gly 2415	2420	2425	7599
10	GAT TTT GGC ACA GCA TTC CCT GAG ATC CCC GTG GAG TTC CTC CAG GAA Asp Phe Gly Thr Ala Phe Pro Glu Ile Pro Val Glu Phe Leu Gln Glu 2430	2435	2440	7647
	AAG GAA GTC TTT AAG GAG TTC ATC TAC CGC ATC AAC ACA CTA GGC TGG Lys Glu Val Phe Lys Glu Phe Ile Tyr Arg Ile Asn Thr Leu Gly Trp 2445	2450	2455	7695
15	ACC AGT CGT ACT CAG TTT GAA GAA ACT TGG GCC ACC CTC CTT GGT GTC Thr Ser Arg Thr Gln Phe Glu Glu Thr Trp Ala Thr Leu Leu Gly Val 2465	2470	2475	7743
	CTG GTG ACG CAG CCC CTC GTG ATG GAG CAG GAG GAG AGC CCA CCA GAA Leu Val Thr Gln Pro Leu Val Met Glu Gln Glu Glu Ser Pro Pro Glu 2480	2485	2490	7791
20	GAA GAC ACA GAG AGG ACC CAG ATC AAC GTC CTG GCC GTG CAG GCC ATC Glu Asp Thr Glu Arg Thr Gln Ile Asn Val Leu Ala Val Gln Ala Ile 2495	2500	2505	7839
	ACC TCA CTG GTG CTC AGT GCA ATG ACT GTG CCT GTG GCC GGC AAC CCA Thr Ser Leu Val Leu Ser Ala Met Thr Val Pro Val Ala Gly Asn Pro 2510	2515	2520	7887
25	GCT GTA AGC TGC TTG GAG CAG CAG CCC CGG AAC AAG CCT CTG AAA GCT Ala Val Ser Cys Leu Glu Gln Gln Pro Arg Asn Lys Pro Leu Lys Ala 2525	2530	2535	7935
	CTC GAC ACC AGG TTT GGG AGG AAG CTG AGC ATT ATC AGA GGG ATT GTG Leu Asp Thr Arg Phe Gly Arg Lys Leu Ser Ile Ile Arg Gly Ile Val 2545	2550	2555	7983
30	GAG CAA GAG ATT CAA GCA ATG GTT TCA AAG AGA GAG AAT ATT GCC ACC Glu Gln Glu Ile Gln Ala Met Val Ser Lys Arg Glu Asn Ile Ala Thr 2560	2565	2570	8031
	CAT CAT TTA TAT CAG GCA TGG GAT CCT GTC CCT TCT CTG TCT CCG GCT His His Leu Tyr Gln Ala Trp Asp Pro Val Pro Ser Leu Ser Pro Ala 2575	2580	2585	8079
35	ACT ACA GGT GCC CTC ATC AGC CAC GAG AAG CTG CTG CTA CAG ATC AAC Thr Thr Gly Ala Leu Ile Ser His Glu Lys Leu Leu Gln Ile Asn 2590	2595	2600	8127
	CCC GAG CGG GAG CTG GGG AGC ATG AGC TAC AAA CTC GGC CAG GTG TCC Pro Glu Arg Glu Leu Gly Ser Met Ser Tyr Lys Leu Gly Gln Val Ser 2605	2610	2615	8175
40	ATA CAC TCC GTG TGG CTG GGG AAC AGC ATC ACA CCC CTG AGG GAG GAG Ile His Ser Val Trp Leu Gly Asn Ser Ile Thr Pro Leu Arg Glu Glu 2625	2630	2635	8223
	GAA TGG GAC GAG GAA GAG GAG GAG GAG GCC GAC GCC CCT GCA CCT TCG Glu Trp Asp Glu Glu Glu Glu Ala Asp Ala Pro Ala Pro Ser 2640	2645	2650	8271
45	TCA CCA CCC ACG TCT CCA GTC AAC TCC AGG AAA CAC CGG GCT GGA GTT Ser Pro Pro Thr Ser Pro Val Asn Ser Arg Lys His Arg Ala Gly Val 2655	2660	2665	8319
	GAC ATC CAC TCC TGT TCG CAG TTT TTG CTT GAG TTG TAC AGC CGC TGG Asp Ile His Ser Cys Ser Gln Phe Leu Leu Glu Leu Tyr Ser Arg Trp 55			8367

	2670	2675	2680	
5	ATC CTG CCG TCC AGC TCA GCC AGG AGG ACC CCG GCC ATC CTG ATC AGT Ile Leu Pro Ser Ser Ser Ala Arg Arg Thr Pro Ala Ile Leu Ile Ser 2685 2690 2695 2700			8415
10	GAG GTG GTC AGA TCC CTT CTA GTG GTC TCA GAC TTG TTC ACC GAG CGC Glu Val Val Arg Ser Leu Leu Val Val Ser Asp Leu Phe Thr Glu Arg 2705 2710 2715			8463
15	AAC CAG TTT GAG CTG ATG TAT GTG ACG CTG ACA GAA CTG CGA AGG GTG Asn Gln Phe Glu Leu Met Tyr Val Thr Leu Thr Glu Leu Arg Arg Val 2720 2725 2730			8511
20	CAC CCT TCA GAA GAC GAG ATC CTC GCT CAG TAC CTG GTG CCT GCC ACC His Pro Ser Glu Asp Glu Ile Leu Ala Gln Tyr Leu Val Pro Ala Thr 2735 2740 2745			8559
25	TGC AAG GCA GCT GCC GTC CTT GGG ATG GAC AAG GCC GTG GCG GAG CCT Cys Lys Ala Ala Ala Val Leu Gly Met Asp Lys Ala Val Ala Glu Pro 2750 2755 2760			8607
30	GTC AGC CGC CTG CTG GAG AGC ACG CTC AGG AGC AGC CAC CTG CCC AGC Val Ser Arg Leu Leu Glu Ser Thr Leu Arg Ser Ser His Leu Pro Ser 2765 2770 2775 2780			8655
35	AGG GTT GGA GCC CTG CAC GGC ATC CTC TAT GTG CTG GAG TGC GAC CTG Arg Val Gly Ala Leu His Gly Ile Leu Tyr Val Leu Glu Cys Asp Leu 2785 2790 2795			8703
40	CTG GAC GAC ACT GCC AAG CAG CTC ATC CCG GTC ATC AGC GAC TAT CTC Leu Asp Asp Thr Ala Lys Gln Leu Ile Pro Val Ile Ser Asp Tyr Leu 2800 2805 2810			8751
45	CTC TCC AAC CTG AAA GGG ATC GCC CAC TGC GTG AAC ATT CAC AGC CAG Leu Ser Asn Leu Lys Gly Ile Ala His Cys Val Asn Ile His Ser Gln 2815 2820 2825			8799
50	CAG CAC GTA CTG GTC ATG TGT GCC ACT GCG TTT TAC CTC ATT GAG AAC Gln His Val Leu Val Met Cys Ala Thr Ala Phe Tyr Leu Ile Glu Asn 2830 2835 2840			8847
55	TAT CCT CTG GAC GTA GGG CCG GAA TTT TCA GCA TCA ATA ATA CAG ATG Tyr Pro Leu Asp Val Gly Pro Glu Phe Ser Ala Ser Ile Ile Gln Met 2845 2850 2855 2860			8895
	TGT GGG GTG ATG CTG TCT GGA AGT GAG GAG TCC ACC CCC TCC ATC ATT Cys Gly Val Met Leu Ser Gly Ser Glu Glu Ser Thr Pro Ser Ile Ile 2865 2870 2875			8943
	TAC CAC TGT GCC CTC AGA GGC CTG GAG CGC CTC CTG CTC TCT GAG CAG Tyr His Cys Ala Leu Arg Gly Leu Glu Arg Leu Leu Ser Glu Gln 2880 2885 2890			8991
	CTC TCC CGC CTG GAT GCA GAA TCG CTG GTC AAG CTG AGT GTG GAC AGA Leu Ser Arg Leu Asp Ala Glu Ser Leu Val Lys Leu Ser Val Asp Arg 2895 2900 2905			9039
	GTG AAC GTG CAC AGC CCG CAC CGG GCC ATG GCG GCT CTG GGC CTG ATG Val Asn Val His Ser Pro His Arg Ala Met Ala Ala Leu Gly Leu Met 2910 2915 2920			9087
	CTC ACC TGC ATG TAC ACA GGA AAG GAG AAA GTC AGT CCG GGT AGA ACT Leu Thr Cys Met Tyr Thr Gly Lys Glu Lys Val Ser Pro Gly Arg Thr 2925 2930 2935 2940			9135
	TCA GAC CCT AAT CCT GCA GCC CCC GAC AGC GAG TCA GTG ATT GTT GCT Ser Asp Pro Asn Pro Ala Ala Pro Asp Ser Glu Ser Val Ile Val Ala 2945 2950 2955			9183
	ATG GAG CGG GTA TCT GTT CTT TTT GAT AGG ATC AGG AAA GGC TTT CCT Met Glu Arg Val Ser Val Leu Phe Asp Arg Ile Arg Lys Gly Phe Pro			9231

	2960	2965	2970	
5	TGT GAA GCC AGA GTG GTG GCC AGG ATC CTG CCC CAG TTT CTA GAC GAC Cys Glu Ala Arg Val Val Ala Arg Ile Leu Pro Gln Phe Leu Asp Asp 2975 2980 2985			9279
10	TTC TTC CCA CCC CAG GAC ATC ATG AAC AAA GTC ATC GGA GAG TTT CTG Phe Phe Pro Pro Gln Asp Ile Met Asn Lys Val Ile Gly Glu Phe Leu 2990 2995 3000			9327
15	TCC AAC CAG CAG CCA TAC CCC CAG TTC ATG GCC ACC GTG GTG TAT AAG Ser Asn Gln Gln Pro Tyr Pro Gln Phe Met Ala Thr Val Val Tyr Lys 3005 3010 3015 3020			9375
20	GTG TTT CAG ACT CTG CAC AGC ACC GGG CAG TCG TCC ATG GTC CGG GAC Val Phe Gln Thr Leu His Ser Thr Gly Gln Ser Ser Met Val Arg Asp 3025 3030 3035			9423
25	TGG GTC ATG CTG CTC TCC AAC TTC ACG CAG AGG GCC CCG GTC GCC Trp Val Met Leu Ser Leu Ser Asn Phe Thr Gln Arg Ala Pro Val Ala 3040 3045 3050			9471
30	ATG GCC ACG TGG AGC CTC TCC TGC TTC TTT GTC AGC GCG TCC ACC AGC Met Ala Thr Trp Ser Leu Ser Cys Phe Phe Val Ser Ala Ser Thr Ser 3055 3060 3065			9519
35	CCG TGG GTC GCG GCG ATC CTC CCA CAT GTC ATC AGC AGG ATG GGC AAG Pro Trp Val Ala Ala Ile Leu Pro His Val Ile Ser Arg Met Gly Lys 3070 3075 3080			9567
40	CTG GAG CAG GTG GAC GTG AAC CTT TTC TGC CTG GTC GCC ACA GAC TTC Leu Glu Gln Val Asp Val Asn Leu Phe Cys Leu Val Ala Thr Asp Phe 3085 3090 3095 3100			9615
45	TAC AGA CAC CAG ATA GAG GAG GAG CTC GAC CGC AGG GCC TTC CAG TCT Tyr Arg His Gln Ile Glu Glu Leu Asp Arg Arg Ala Phe Gln Ser 3105 3110 3115			9663
50	GTG CTT GAG GTG GTT GCA GCC CCA GGA AGC CCA TAT CAC CGG CTG CTG Val Leu Glu Val Val Ala Ala Pro Gly Ser Pro Tyr His Arg Leu Leu 3120 3125 3130			9711
55	ACT TGT TTA CGA AAT GTC CAC AAG GTC ACC ACC TGC T GAGCGCCATG Thr Cys Leu Arg Asn Val His Lys Val Thr Thr Cys 3135 3140			9758
	GTGGGAGAGA CTGTGAGGCG GCAGCTGGGG CGGGAGCCTT TGGAAAGTCTG TGCCCTTGTG CCCTGCCTCC ACCGAGCCAG CTTGGTCCCT ATGGGCTTCC GCACATGCCG CGGGCGGCCA GGCAACGTGC GTGTCTCTGC CATGTGGCAG AAGTGCCTT TGTGGCAGTG GCCAGGCAGG GAGTGTCTGC AGTCCTGGTG GGGCTGAGCC TGAGGCCTTC CAGAAAGCAG GAGCAGCTGT GCTGCACCCC ATGTGGGTGA CCAGGTCCTT TCTCCTGATA GTCACCTGCT GGTGTTGCC AGGTGCAAGC TGCTCTTGCA TCTGGGCCAG AAGTGCCTCC TCCTGCAGGC TGGCTGTTGG CCCCCTTGCT GTCTGCAGT AGAAGGTGCC GTGAGCAGGC TTTGGGAACA CTGGCCTGGG TCTCCCTGGT GGGGTGTGCA TGCCACGCC CGTGTCTGGA TGCACAGATG CCATGGCCTG TGCTGGGCCA GTGGCTGGGG GTGCTAGACA CCCGGCACCA TTCTCCCTTC TCTCTTTCT TCTCAGGATT TAAAATTTAA TTATATCAGT AAAGAGATTA ATTTAACGT AAAAAAAA AAAAAAA			9818 9878 9938 9998 10058 10118 10178 10238 10298 10358 10366

(2) INFORMATION FOR SEQ ID NO:6:

[0151]

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3144 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Ala Thr Leu Glu Lys Leu Met Lys Ala Phe Glu Ser Leu Lys Ser  
 1 5 10 15  
 Phe Gln  
 5 20 25 30  
 Gln Gln Gln Gln Gln Gln Pro Pro Pro Pro Pro Pro Pro Pro Pro  
 35 40 45  
 Pro Pro Pro Gln Leu Pro Gln Pro Pro Gln Ala Gln Pro Leu Leu  
 10 50 55 60  
 Pro Gln Pro Gln Pro Pro Pro Pro Pro Pro Pro Pro Pro Gly Pro  
 65 70 75 80  
 Ala Val Ala Glu Glu Pro Leu His Arg Pro Lys Lys Glu Leu Ser Ala  
 15 85 90 95  
 Thr Lys Lys Asp Arg Val Asn His Cys Leu Thr Ile Cys Glu Asn Ile  
 100 105 110  
 Val Ala Gln Ser Val Arg Asn Ser Pro Glu Phe Gln Lys Leu Leu Gly  
 20 115 120 125  
 Ile Ala Met Glu Leu Phe Leu Leu Cys Ser Asp Asp Ala Glu Ser Asp  
 130 135 140  
 Val Arg Met Val Ala Asp Glu Cys Leu Asn Lys Val Ile Lys Ala Leu  
 25 145 150 155 160  
 Met Asp Ser Asn Leu Pro Arg Leu Gln Leu Glu Leu Tyr Lys Glu Ile  
 165 170 175  
 Lys Lys Asn Gly Ala Pro Arg Ser Leu Arg Ala Ala Leu Trp Arg Phe  
 30 180 185 190  
 Ala Glu Leu Ala His Leu Val Arg Pro Gln Lys Cys Arg Pro Tyr Leu  
 195 200 205  
 Val Asn Leu Leu Pro Cys Leu Thr Arg Thr Ser Lys Arg Pro Glu Glu  
 210 215 220  
 Ser Val Gln Glu Thr Leu Ala Ala Val Pro Lys Ile Met Ala Ser  
 35 225 230 235 240  
 Phe Gly Asn Phe Ala Asn Asp Asn Glu Ile Lys Val Leu Leu Lys Ala  
 245 250 255  
 Phe Ile Ala Asn Leu Lys Ser Ser Pro Thr Ile Arg Arg Thr Ala  
 40 260 265 270  
 Ala Gly Ser Ala Val Ser Ile Cys Gln His Ser Arg Arg Thr Gln Tyr  
 275 280 285  
 Phe Tyr Ser Trp Leu Leu Asn Val Leu Leu Gly Leu Leu Val Pro Val  
 45 290 295 300  
 Glu Asp Glu His Ser Thr Leu Leu Ile Leu Gly Val Leu Leu Thr Leu  
 305 310 315 320  
 Arg Tyr Leu Val Pro Leu Leu Gln Gln Val Lys Asp Thr Ser Leu  
 50 325 330 335  
 Lys Gly Ser Phe Gly Val Thr Arg Lys Glu Met Glu Val Ser Pro Ser

	340	345	350
	Ala Glu Gln Leu Val Gln Val Tyr	Glu Leu Thr Leu His His Thr Gln	
5	355 360	365	
	His Gln Asp His Asn Val Val Thr Gly	Ala Leu Glu Leu Leu Gln Gln	
	370 375	380	
	Leu Phe Arg Thr Pro Pro Pro Glu Leu Leu Gln Thr Leu Thr Ala Val		
	385 390	395	400
10	Gly Gly Ile Gly Gln Leu Thr Ala Ala Lys	Glu Glu Ser Gly Gly Arg	
	405	410	415
	Ser Arg Ser Gly Ser Ile Val Glu Leu Ile Ala Gly Gly Ser Ser		
	420 425	430	
15	Cys Ser Pro Val Leu Ser Arg Lys	Gln Lys Gly Lys Val Leu Leu Gly	
	435	440	445
	Glu Glu Glu Ala Leu Glu Asp Asp Ser Glu Ser Arg Ser Asp Val Ser		
	450 455	460	
20	Ser Ser Ala Leu Thr Ala Ser Val Lys Asp Glu Ile Ser Gly Glu Leu		
	465 470	475	480
	Ala Ala Ser Ser Gly Val Ser Thr Pro Gly Ser Ala Gly His Asp Ile		
	485 490	495	
	Ile Thr Glu Gln Pro Arg Ser Gln His Thr Leu Gln Ala Asp Ser Leu		
	500 505	510	
25	Asp Leu Ala Ser Cys Asp Leu Thr Ser Ser Ala Thr Asp Gly Asp Glu		
	515 520	525	
	Glu Asp Ile Leu Ser His Ser Ser Ser Gln Val Ser Ala Val Pro Ser		
	530 535	540	
30	Asp Pro Ala Met Asp Leu Asn Asp Gly Thr Gln Ala Ser Ser Pro Ile		
	545 550	555	560
	Ser Asp Ser Ser Gln Thr Thr Glu Gly Pro Asp Ser Ala Val Thr		
	565 570	575	
35	Pro Ser Asp Ser Ser Glu Ile Val Leu Asp Gly Thr Asp Asn Gln Tyr		
	580 585	590	
	Leu Gly Leu Gln Ile Gly Gln Pro Gln Asp Glu Asp Glu Ala Thr		
	595 600	605	
	Gly Ile Leu Pro Asp Glu Ala Ser Glu Ala Phe Arg Asn Ser Ser Met		
	610 615	620	
40	Ala Leu Gln Gln Ala His Leu Leu Lys Asn Met Ser His Cys Arg Gln		
	625 630	635	640
	Pro Ser Asp Ser Ser Val Asp Lys Phe Val Leu Arg Asp Glu Ala Thr		
	645 650	655	
45	Glu Pro Gly Asp Gln Glu Asn Lys Pro Cys Arg Ile Lys Gly Asp Ile		
	660 665	670	
	Gly Gln Ser Thr Asp Asp Asp Ser Ala Pro Leu Val His Ser Val Arg		
	675 680	685	
50	Leu Leu Ser Ala Ser Phe Leu Leu Thr Gly Gly Lys Asn Val Leu Val		
	690 695	700	
	Pro Asp Arg Asp Val Arg Val Ser Val Lys Ala Leu Ala Leu Ser Cys		
	705 710	715	720
55	Val Gly Ala Ala Val Ala Leu His Pro Glu Ser Phe Phe Ser Lys Leu		
	725 730	735	

Tyr Lys Val Pro Leu Asp Thr Thr Glu Tyr Pro Glu Glu Gln Tyr Val  
 740 745 750  
 Ser Asp Ile Leu Asn Tyr Ile Asp His Gly Asp Pro Gln Val Arg Gly  
 5 755 760 765  
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 15 Val Ala Ala Pro Gly Ser Pro Tyr His Arg Leu Leu Thr Cys Leu Arg  
 3125 3130 3135  
 Asn Val His Lys Val Thr Thr Cys  
 3140

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### Claims

1. An isolated, purified or recombinant huntingtin polypeptide comprising the amino acid sequence shown in SEQ ID NO:6.
2. An isolated, purified or recombinant nucleic acid molecule comprising a huntingtin nucleic acid molecule encoding a huntingtin polypeptide according to claim 1, or its complementary strand.
3. A nucleic acid molecule according to claim 2, comprising the nucleic acid shown in SEQ ID NO:5.
4. A nucleic acid molecule according to claim 2 or claim 3, comprising a transcriptional control region operably linked to said huntingtin nucleic acid molecule.
5. A vector comprising a nucleic acid molecule according to any of claims 2 to 4.
6. A vector according to claim 5, wherein the nucleic acid molecule is operably linked to transcriptional and/or translational expression signals.
7. A host cell transformed or transfected with a vector according to claim 5 or claim 6.
8. An antibody specific for huntingtin polypeptide as claimed in claim 1.
9. A hybridoma which produces an antibody according to claim 8.
- 45 10. A method of detecting the presence of, or predisposition to develop, Huntington's disease in a subject, the method comprising
  - (a) evaluating the characteristics of huntingtin nucleic acid in a sample from the subject, wherein the evaluation comprises detecting the huntingtin (CAG)<sub>n</sub> region shown in SEQ ID NO:5 in the sample; and
  - (b) comparing the characteristics found in (a) with a similar analysis from an individual with no family history of Huntington's disease, where the nucleic acid has from 11 to 34 (CAG) repeats, the presence of, or predisposition to develop, Huntington's disease being indicated if those characteristics in the huntingtin (CAG)<sub>n</sub>

region differ.

11. A method according to claim 10, wherein the characteristics of huntingtin nucleic acid are evaluated by Southern blot, northern blot, or polymerase chain reaction analysis.

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12. The use of:

- (a) a nucleic acid molecule according to claim 2, 3 or 4 or a vector according to claim 5 or claim 6;
- (b) a polypeptide according to claim 1; and/or
- (c) a host cell according to claim 7

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in the preparation of a medicament.

13. The use according to claim 12, wherein the medicament is for treating, delaying or preventing a neurodegenerative disorder.

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14. The use according to claim 12 or claim 13, wherein the medicament is for gene therapy.

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15. The use according to claim 12, 13 or 14, wherein the medicament is for treating, preventing or delaying Huntingdon's disease.

16. The use according to any of claims 12 to 15 wherein the nucleic acid has from 11 to 34 (CAG) repeats and/or the polypeptide has from 11 to 34 Gln repeats, said repeats being consecutive.

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17. A diagnostic and/or immunoassay kit comprising at least one container and;

- (a) a nucleic acid molecule according to claim 2, 3 or 4, optionally labelled; or
- (b) an antibody according to claim 8, optionally labelled.

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18. A pharmaceutical composition comprising:

- (a) a nucleic acid molecule according to claim 2, 3 or 4 or a vector according to claim 5 or claim 6;
- (b) a polypeptide according to claim 1; and/or
- (c) a host cell according to claim 7

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in admixture with pharmaceutically acceptable carrier.

19. A process for the preparation of a polypeptide according to claim 1, the process comprising culturing a host cell according to claim 7 under conditions whereby the polypeptide is expressed, and purifying or isolating the polypeptide.

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#### Patentansprüche

1. Isoliertes, gereinigtes oder rekombiniertes Huntingtin-Polypeptid, das die unter SEQ ID NO:6 wiedergegebene Aminosäuresequenz enthält.

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2. Isoliertes, gereinigtes oder rekombiniertes Nucleinsäuremolekül, das ein Huntingtin-Nucleinsäuremolekül enthält, das ein Huntingtin-Polypeptid gemäß Anspruch 1 oder seinen Zusatzstrang codiert.

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3. Nucleinsäuremolekül gemäß Anspruch 2, das die unter SEQ ID NO:5 wiedergegebene Nucleinsäure enthält.

4. Nucleinsäuremolekül gemäß Anspruch 2 oder 3, das einen Transkriptionskontrollbereich aufweist, der operativ mit dem Huntingtin-Nucleinsäuremolekül verbunden ist.

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5. Vektor, der ein Nucleinsäuremolekül gemäß einem der Ansprüche 2 bis 4 aufweist.

6. Vektor gemäß Anspruch 5, wobei das Nucleinsäuremolekül operativ mit Transkriptions- und/oder Translationsausdruckssignalen verbunden ist.

7. Wirtszelle, die mit einem Vektor gemäß Anspruch 5 oder Anspruch 6 transformiert oder transfektiert wird.
8. Antikörper, der für das Huntingtin-Polypeptid gemäß Anspruch 1 spezifisch ist.
- 5 9. Hybridoma, das einen Antikörper gemäß Anspruch 8 erzeugt.
10. Verfahren zum Erfassen der Gegenwart der Huntington-Erkrankung oder der Neigung zur Entwicklung der Huntington-Erkrankung in einer Person, wobei das Verfahren Folgendes beinhaltet:
  - 10 (a) Evaluierung der Charakteristika der Huntingtin-Nucleinsäure in einer der Person entnommenen Probe, wobei die Evaluierung das Erfassen des unter SEQ ID NO:5 dargestellten Huntingtin (CAG)-Bereichs in der Probe einschließt, und
  - (b) Vergleichen der in (a) festgestellten Charakteristika mit einer ähnlichen Analyse, die bei einer Person durchgeführt wurde, in deren Familie keine Huntington-Erkrankung vorliegt und bei der die Nucleinsäure 11 bis 34 (CAG) Wiederholungen aufweist, wobei die Gegenwart der Huntington-Erkrankung oder die Neigung zur Entwicklung der Huntington-Erkrankung angezeigt wird, wenn sich diese Charakteristika im Huntingtin (CAG)-Bereich unterscheiden.
11. Verfahren gemäß Anspruch 10, wobei die Charakteristika der Huntingtin-Nucleinsäure durch Southern-Blot-Analyse, Northern-Blot-Analyse oder eine Polymerase-Kettenreaktionsanalyse evaluiert werden.
- 20 12. Verwendung
  - 25 (a) eines Nucleinsäuremoleküls gemäß Anspruch 2, 3 oder 4 oder eines Vektors gemäß Anspruch 5 oder Anspruch 6;
  - (b) eines Polypeptids gemäß Anspruch 1 und/oder
  - (c) einer Wirtszelle gemäß Anspruch 7

30 bei der Herstellung eines Medikaments.

- 30 13. Verwendung gemäß Anspruch 12, wobei das Medikament zur Behandlung, Verzögerung oder Vermeidung einer neurodegenerativen Erkrankung eingesetzt wird.
- 35 14. Verwendung gemäß Anspruch 12 oder Anspruch 13, wobei das Medikament für eine Gentherapie eingesetzt wird.
- 15. Verwendung gemäß Anspruch 12, 13 oder 14, wobei das Medikament zur Behandlung, Vermeidung oder Verzögerung der Huntington-Erkrankung dient.
- 40 16. Verwendung gemäß einem der Ansprüche 12 bis 15, wobei die Nucleinsäure 11 bis 34 (CAG) Wiederholungen aufweist und/oder das Polypeptid 11 bis 34 Gln-Wiederholungen aufweist, wobei die Wiederholungen aufeinander folgen.
- 17. Diagnostische und/oder Immunoassay-Ausrüstung, die mindestens einen Behälter und
- 45 (a) ein, gegebenenfalls markiertes, Nucleinsäuremolekül gemäß Anspruch 2, 3 oder 4 oder  
(b) einen gegebenenfalls markierten Antikörper gemäß Anspruch 8 enthält.
- 18. Pharmazeutische Zusammensetzung, die Folgendes enthält:
  - 50 (a) ein Nucleinsäuremolekül gemäß Anspruch 2, 3 oder 4 oder einen Vektor gemäß Anspruch 5 oder Anspruch 6;
  - (b) ein Polypeptid gemäß Anspruch 1 und/oder
  - (c) eine Wirtszelle gemäß Anspruch 7
- 55 in einer Mischung mit einem pharmazeutisch akzeptablen Träger.
- 19. Verfahren zur Herstellung eines Polypeptids gemäß Anspruch 1, wobei das Verfahren das Kultivieren einer Wirtszelle gemäß Anspruch 7 unter Bedingungen, bei denen das Polypeptid exprimiert wird, und das Reinigen oder

Isolieren des Polypeptids aufweist.

**Revendications**

- 5 1. Polypeptide huntingtin isolé, purifié ou recombinant comprenant la séquence d'acides aminés présentée dans SEQ ID n° 6.
- 10 2. Molécule d'acide nucléique isolé, purifié ou recombinant comprenant une molécule d'acide nucléique huntingtin codant pour un polypeptide huntingtin suivant la revendication 1 ou son brin complémentaire.
- 15 3. Molécule d'acide nucléique suivant la revendication 2, comprenant l'acide nucléique présenté dans SEQ ID n° 5.
4. Molécule d'acide nucléique suivant la revendication 2 ou la revendication 3, comprenant une région de contrôle de transcription liée de manière fonctionnelle à ladite molécule d'acide nucléique huntingtin.
5. Vecteur comprenant une molécule d'acide nucléique suivant l'une quelconque des revendications 2 à 4.
- 20 6. Vecteur suivant la revendication 5, dans lequel la molécule d'acide nucléique est liée de manière fonctionnelle à des signaux d'expression de transcription et/ou de traduction.
7. Cellule hôte transformée ou transfectée avec un vecteur suivant la revendication 5 ou la revendication 6.
- 25 8. Anticorps spécifique pour un polypeptide huntingtin suivant la revendication 1.
9. Hybridome qui produit un anticorps suivant la revendication 8.
- 30 10. Procédé de détection de la présence de la maladie de Huntington ou d'une prédisposition à la développer chez un sujet, le procédé comprenant
  - (a) l'évaluation des caractéristiques de l'acide nucléique huntingtin dans un échantillon du sujet, dans lequel l'évaluation comprend une détection de la région huntingtin (CAG)<sub>a</sub> présentée dans SEQ ID n° 5 dans l'échantillon; et
  - (b) la comparaison des caractéristiques trouvées en (a) avec une analyse similaire provenant d'un individu sans antécédents familiaux de maladie de Huntington, où l'acide nucléique présente de 11 à 34 répétitions (CAG), la présence de la maladie de Huntington, ou une prédisposition à la développer, étant indiquée si ces caractéristiques dans la région huntingtin (CAG)<sub>n</sub> diffèrent.
- 35 11. Procédé suivant la revendication 10, dans lequel les caractéristiques de l'acide nucléique huntingtin sont évaluées par buvardage de Southern, buvardage de Northern, ou une analyse par réaction de polymérase en chaîne.
- 40 12. Utilisation :
  - (a) d'une molécule d'acide nucléique suivant la revendication 2, 3 ou 4 ou d'un vecteur suivant la revendication 5 ou la revendication 6;
  - (b) d'un polypeptide suivant la revendication 1; et/ou
  - (c) d'une cellule hôte suivant la revendication 7 dans la préparation d'un médicament.
- 45 13. Utilisation suivant la revendication 12, dans laquelle le médicament est destiné à traiter, à retarder ou à empêcher un trouble neurodégénératif.
- 50 14. Utilisation suivant la revendication 12 ou la revendication 13, dans laquelle le médicament a pour but une thérapie génique.
- 55 15. Utilisation suivant la revendication 12, 13 ou 14, dans laquelle le médicament est destiné à traiter, empêcher ou retarder la maladie d'Huntington.
16. Utilisation suivant l'une quelconque des revendications 12 à 15, dans laquelle l'acide nucléique présente de 11 à

34 répétitions (CAG) et/ou le polypeptide présente de 11 à 34 répétitions Gln, lesdites répétitions étant successives.

17. Trousse pour diagnostic et/ou essais immunologiques comprenant au moins un récipient et ;

5 (a) une molécule d'acide nucléique suivant la revendication 2, 3 ou 4, facultativement marquée ; ou  
(b) un anticorps suivant la revendication 8, facultativement marqué.

18. Composition pharmaceutique contenant :

10 (a) une molécule d'acide nucléique suivant la revendication 2, 3 ou 4 ou un vecteur suivant la revendication 5 ou la revendication 6.  
(b) un polypeptide suivant la revendication 1 ; et/ou  
(c) une cellule hôte suivant la revendication 7

15 en mélange avec un excipient pharmaceutiquement acceptable.

19. Procédé de préparation d'un polypeptide suivant la revendication 1, le procédé comprenant la culture d'une cellule hôte suivant la revendication 7 dans des conditions par lesquelles le polypeptide est exprimé, et la purification ou l'isolement du polypeptide.

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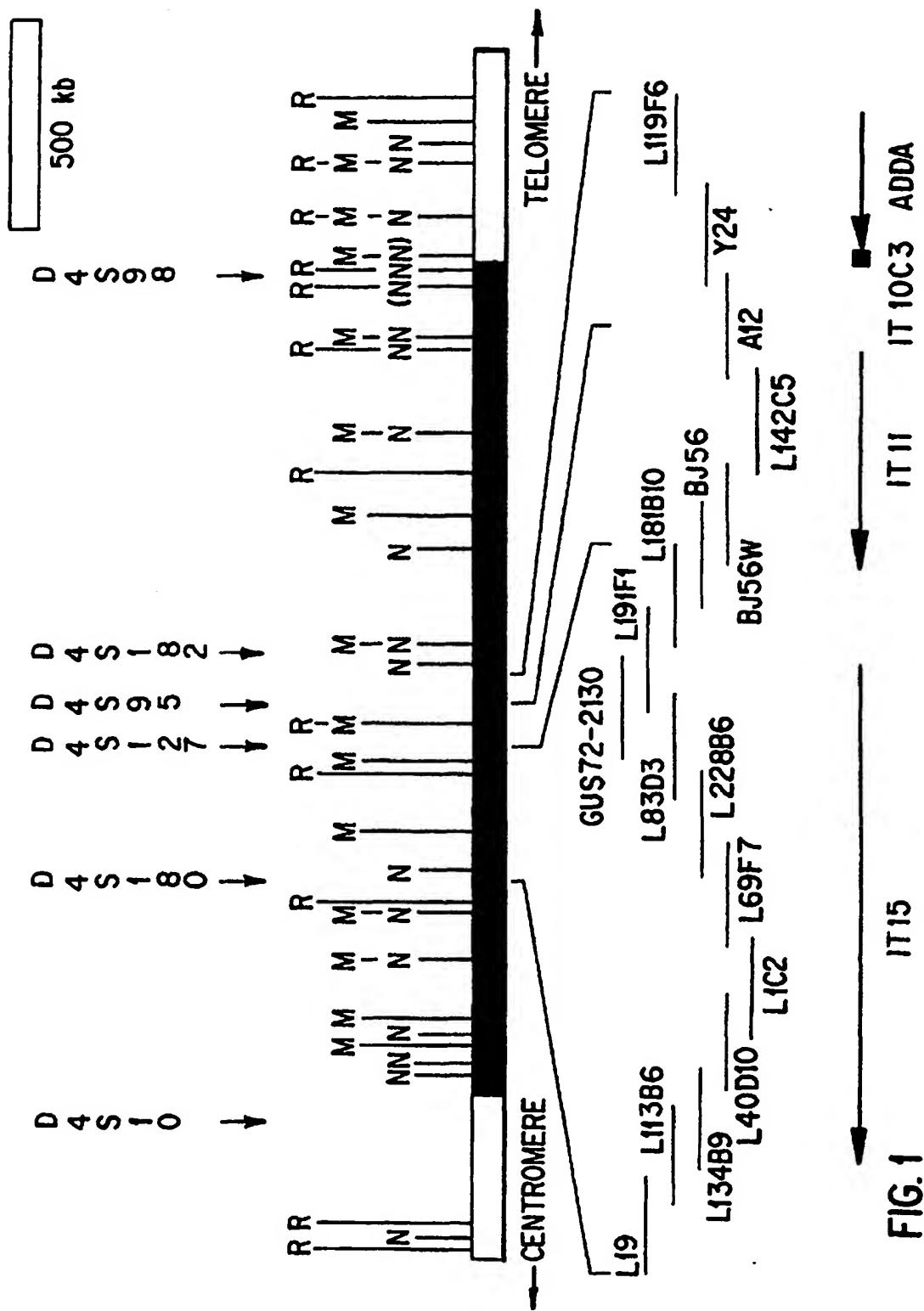
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—  
FIG.

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1 2 3



— 28S



— 18S

FIG. 2

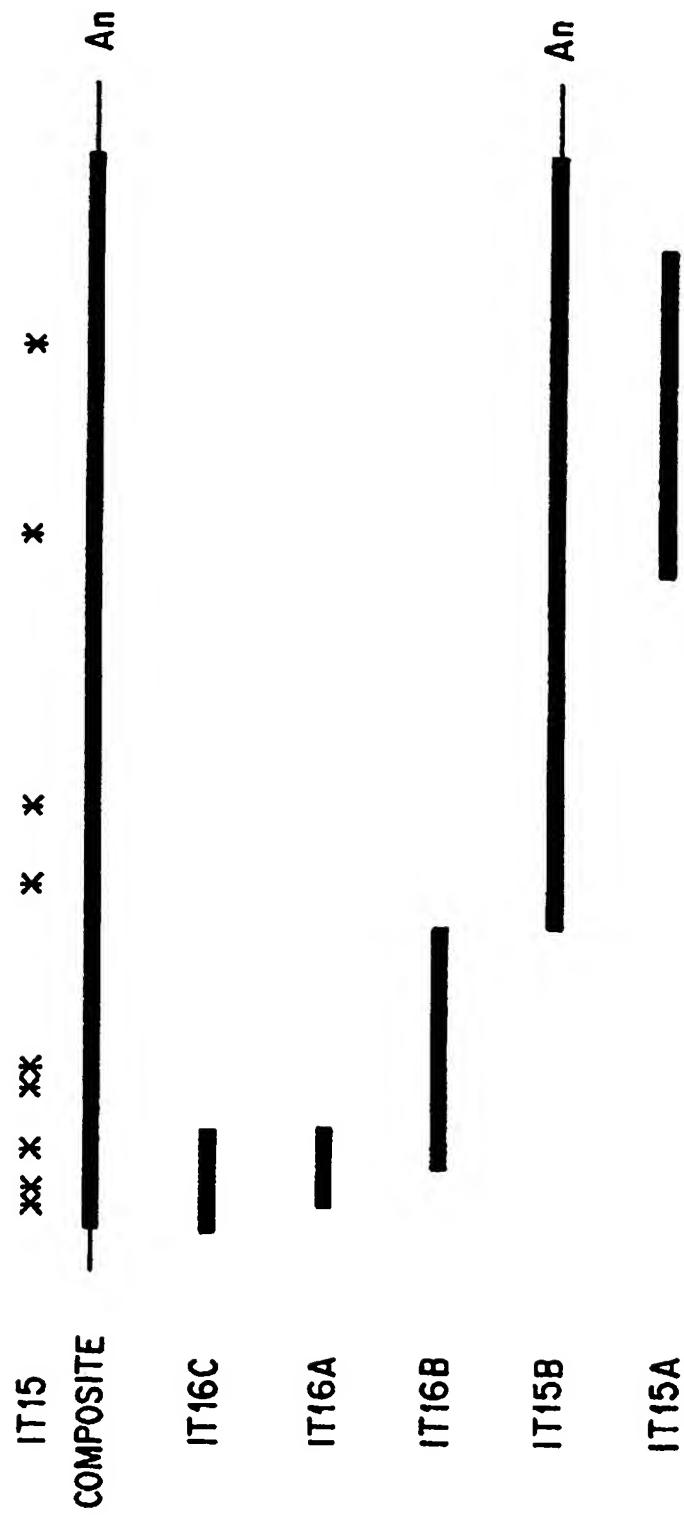


FIG. 3

TTCCTCTGTC	AGGCAGAAC	TGCCGGGCA	GGGGGGGCT	GGTTCCCTGC	CCAGCCATTG	60
GCACAGTCG	CAGGCTAGGG	CTCTCAATCA	TGCTGGCCGG	CGTGGCCCCG	CCTCCGCCGG	120
CGCGGGCCCCG	CCTCCGCCGG	CGGACGTCTG	GGACCCAAGG	CGCCGTGGGG	GCTGCCGGGA	180
CGGGTCCAAG	ATGGACGGCC	GCTCAGGTTC	TGCTTTTACC	TGCGGGCCAG	AGCCCCATTG	240
ATTGCCCGGG	TGCTGAGGG	CGCGCGGAGT	CGGGCGGAGG	CCTCCGGGGA	CTGCCGTGCC	300
GGCCGGGAGA	CGGCC	ATG GCG ACC CTG GAA AAG CTG ATG AAG GCC TTC GAC				351
		Met Ala Thr Leu Glu Lys Leu Met Lys Ala Phe Glu				
	1		5		10	
TCC CTC AAG TCC TTC CAG CAG CAG CAG CAG CAG CAG CAG CAG						399
Ser Leu Lys Ser Phe Gln						
15		20		25		
CAG CAG CAG CAG CAG CAG CAG CAG CAA CAG COG CCA CCC CCC						.447
Gln Pro Pro Pro						
30		35		40		
CGG CGG CGG CGG CGG CCT CCT CAG CTT CCT CAG CGG CGG CGG CGG CAG GCA						495
Pro Pro Pro Pro Pro Pro Gln Leu Pro Gln Pro Pro Pro Gln Ala						
45		50		55		60
CAG CGG CTG CTG CCT CAG CGG CAG CCC CGG CGG CGG CCC CGG CGG						543
Gln Pro Leu Leu Pro Gln Pro Gln Pro Pro Pro Pro Pro Pro Pro						
65		70		75		
CCA CCC GGC CCG GCT GTG GCT GAG GAC CGG CTG CAC CGA CCA AAG AAA						591
Pro Pro Gly Pro Ala Val Ala Glu Glu Pro Leu His Arg Pro Lys Lys						
80		85		90		
GAA CTT TCA GCT ACC AAG AAA GAC CGT GTG AAT CAT TGT CTG ACA ATA						639
Glu Leu Ser Ala Thr Lys Lys Asp Arg Val Asn His Cys Leu Thr Ile						
95		100		105		
TGT GAA AAC ATA GTG GCA CAG TCT GTC AGA AAT TCT CCA GAA TTT CAG						687
Cys Glu Asn Ile Val Ala Gln Ser Val Arg Asn Ser Pro Glu Phe Gln						
110		115		120		

FIG. 4A

AAA CTT CTG GCC ATC GCT ATG GAA CTT TTT CTG CTG TGC ACT GAT GAC Lys Leu Leu Gly Ile Ala Met Glu Leu Phe Leu Leu Cys Ser Asp Asp 125 130 135 140	735
GCA GAG TCA GAT GTC AGG ATG GTG GCT GAC GAA TGC CTC AAC AAA GTT Ala Glu Ser Asp Val Arg Met Val Ala Asp Glu Cys Leu Asn Lys Val 145 150 155	783
ATC AAA GCT TTG ATG GAT TCT AAT CTT CCA AGG TTA CAG CTC GAG CTC Ile Lys Ala Leu Met Asp Ser Asn Leu Pro Arg Leu Gln Leu Glu Leu 160 165 170	831
TAT AAG GAA ATT AAA AAG AAT GGT GCC CCT CGG ACT TTG CGT GCT GCC Tyr Lys Glu Ile Lys Lys Asn Gly Ala Pro Arg Ser Leu Arg Ala Ala 175 180 185	879
CTG TGG AGG TTT GCT GAG CTG GCT CAC CTG GTT CGG CCT CAG AAA TGC Leu Trp Arg Phe Ala Glu Leu Ala His Leu Val Arg Pro Gln Lys Cys 190 195 200	927
AGG CCT TAC CTG GTG AAC CTT CTG CGG TGC ACT CGA ACA AGC AAG Arg Pro Tyr Leu Val Asn Leu Leu Pro Cys Leu Thr Arg Thr Ser Lys 205 210 215 220	975
AGA CCC GAA GAA TCA GTC CAG GAC ACC TTG GCT GCA GCT GTT CCC AAA Arg Pro Glu Glu Ser Val Gln Glu Thr Leu Ala Ala Ala Val Pro Lys 225 230 235	1023
ATT ATG GCT TCT TTT GGC AAT TTT GCA AAT GAC AAT GAA ATT AAG GTT Ile Met Ala Ser Phe Gly Asn Phe Ala Asn Asp Asn Glu Ile Lys Val 240 245 250	1071
TTC TTA AAG GCC TTC ATA CGG AAC CTG AAG TCA AGC TCC CCC ACC ATT Leu Leu Lys Ala Phe Ile Ala Asn Leu Lys Ser Ser Ser Pro Thr Ile 255 260 265	1119
CGG CGG ACA CGG GCT GGA TCA GCA GTG AGC ATC TGC CAG CAC TCA AGA Arg Arg Thr Ala Ala Gly Ser Ala Val Ser Ile Cys Gln His Ser Arg 270 275 280	1167

FIG.4B

AGG ACA CAA TAT TTC TAT AGT TGG CTA CTA AAT GTG CTC TTA GGC TTA Arg Thr Gln Tyr Phe Tyr Ser Trp Leu Leu Asn Val Leu Leu Gly Leu 285 290 295 300	1215
CTC GTT CCT GTC GAG GAT GAA CAC TCC ACT CTG CTG ATT CTT GGC GTG Leu Val Pro Val Glu Asp Glu His Ser Thr Leu Leu Ile Leu Gly Val 305 310 315	1263
CTG CTC ACC CTG AGG TAT TTG GTG CCC TTG CTG CAG CAG CAG GTC AAG Leu Leu Thr Leu Arg Tyr Leu Val Pro Leu Leu Gln Gln Val Lys 320 325 330	1311
GAC ACA AGC CTG AAA GGC AGC TTC GGA GTG ACA AGG AAA GAA ATG GAA Asp Thr Ser Leu Lys Gly Ser Phe Gly Val Thr Arg Lys Glu Met Glu 335 340 345	1359
GTC TCT CCT TCT GCA GAG CAG CTT GTC CAG GTT TAT GAA CTG ACC TTA Val Ser Pro Ser Ala Glu Gln Leu Val Gln Val Tyr Glu Leu Thr Leu 350 355 360	1407
CAT CAT ACA CAG CAC CAA GAC CAC AAT GTT GTG ACC CGA GCC CTG GAG His His Thr Gln His Gln Asp His Asn Val Val Thr Gly Ala Leu Glu 365 370 375 380	1455
CTG TTG CAG CAG CTC TTC AGA ACG CCT CCA CCC GAG CTT CTG CAA ACC Leu Leu Gln Gln Leu Phe Arg Thr Pro Pro Pro Glu Leu Leu Gln Thr 385 390 395	1503
CTG ACC GCA GTC CGG GGC ATT GGG CAG CTC ACC GCT GCT AAG GAG GAG Leu Thr Ala Val Gly Gly Ile Gly Gln Leu Thr Ala Ala Lys Glu Glu 400 405 410	1551
TCT GGT CGC CGA AGC CGT AGT GGG AGT ATT GTG GAA CTT ATA GCT GGA Ser Gly Gly Arg Ser Arg Ser Gly Ser Ile Val Glu Leu Ile Ala Gly 415 420 425	1599
GGG GGT TCC TCA TGC ACC CCT GTC CTT TCA AGA AAA CAA AAA GGC AAA Gly Gly Ser Ser Cys Ser Pro Val Leu Ser Arg Lys Gln Lys Gly Lys 430 435 440	1647

FIG.4C

GTG CTC TTA GGA GAA GAA GCC TTG GAG GAT GAC TCT GAA TCG AGA Val Leu Leu Gly Glu Glu Glu Ala Leu Glu Asp Asp Ser Glu Ser Arg 445 450 455 460	1695
TCG GAT GTC AGC AGC TCT GCC TTA ACA GCC TCA GTC AAG GAT GAG ATC Ser Asp Val Ser Ser Ser Ala Leu Thr Ala Ser Val Lys Asp Glu Ile 465 470 475	1743
ACT GGA GAG CTG GCT TCT TCA GGG GTT TCC ACT CCA GGG TCA GCA Ser Gly Glu Leu Ala Ala Ser Ser Gly Val Ser Thr Pro Gly Ser Ala 480 485 490	1791
GGT CAT GAC ATC ATC ACA GAA CAG CCA CGG TCA CAG CAC ACA CTG CAG Gly His Asp Ile Ile Thr Glu Gln Pro Arg Ser Gln His Thr Leu Gln 495 500 505	1839
GCG GAC TCA CTG GAT CTG GCC AGC TGT GAC TTG ACA AGC TCT GCC ACT Ala Asp Ser Leu Asp Leu Ala Ser Cys Asp Leu Thr Ser Ser Ala Thr 510 515 520	1887
GAT GGG GAT GAG GAG GAT ATC TTG AGC CAC AGC TCC AGC CAG GTC AGC Asp Gly Asp Glu Glu Asp Ile Leu Ser His Ser Ser Ser Gln Val Ser 525 530 535 540	1935
GCC GTC CCA TCT GAC CCT GCC ATG GAC CTG AAT GAT GGG ACC CAG GCC Ala Val Pro Ser Asp Pro Ala Met Asp Leu Asn Asp Gly Thr Gln Ala 545 550 555	1983
TCG TCG CCC ATC AGC GAC AGC TCC CAG ACC ACC ACC GAA GGG CCT GAT Ser Ser Pro Ile Ser Asp Ser Ser Gln Thr Thr Thr Glu Gly Pro Asp 560 565 570	2031
TCA GCT GTT ACC CCT TCA GAC AGT TCT GAA ATT GTG TTA GAC GGT ACC Ser Ala Val Thr Pro Ser Asp Ser Ser Glu Ile Val Leu Asp Gly Thr 575 580 585	2079
GAC AAC CAG TAT TTG GCC CTG CAG ATT GGA CAG CCC CAG GAT GAA GAT Asp Asn Gln Tyr Leu Gly Leu Gln Ile Gly Gln Pro Gln Asp Glu Asp 590 595 600	2127

FIG.4D

GAG GAA GCC ACA GGT ATT CTT CCT GAT GAA GCC TCG GAG GCC TTC AGG Glu Glu Ala Thr Gly Ile Leu Pro Asp Glu Ala Ser Glu Ala Phe Arg 605 610 615 620	2175
AAC TCT TCC ATG GCC CTT CAA CAG GCA CAT TTA TTG AAA AAC ATG AGT Asn Ser Ser Met Ala Leu Gln Gln Ala His Leu Leu Lys Asn Met Ser 625 630 635	2223
CAC TGC AGG CAG CCT TCT GAC AGC AGT GTT GAT AAA TTT GTG TTG AGA His Cys Arg Gln Pro Ser Asp Ser Ser Val Asp Lys Phe Val Leu Arg 640 645 650	2271
GAT GAA CCT ACT GAA CCG CGT GAT CAA GAA AAC AAG CCT TGC CCC ATC Asp Glu Ala Thr Glu Pro Gly Asp Gln Glu Asn Lys Pro Cys Arg Ile 655 660 665	2319
AAA GGT GAC ATT GGA CAG TCC ACT GAT GAC TCT GCA CCT CTT GTC Lys Gly Asp Ile Gly Gln Ser Thr Asp Asp Asp Ser Ala Pro Leu Val 670 675 680	2367
CAT TCT GTC CGC CTT TTA TCT GCT TCG TTT TTG CTA ACA CGG GGA AAA His Ser Val Arg Leu Leu Ser Ala Ser Phe Leu Leu Thr Gly Gly Lys 685 690 695 700	2415
AAT GTG CTC GTT CCG GAC AGG GAT GTG AGG GTC AGC GTG AAG GCC CTG Asn Val Leu Val Pro Asp Arg Asp Val Arg Val Ser Val Lys Ala Leu 705 710 715	2463
GCC CTC AGC TGT GTG GGA GCA GCT GTG GCC CTC CAC CGG GAA TCT TTC Ala Leu Ser Cys Val Gly Ala Ala Val Ala Leu His Pro Glu Ser Phe 720 725 730	2511
TTC AGC AAA CTC TAT AAA GTT CCT CTT GAC ACC ACG GAA TAC CCT GAC Phe Ser Lys Leu Tyr Lys Val Pro Leu Asp Thr Thr Glu Tyr Pro Glu 735 740 745	2559

FIG.4E

GAA CAG TAT GTC TCA GAC ATC TTG AAC TAC ATC GAT CAT CGA GAC CCA Glu Gln Tyr Val Ser Asp Ile Leu Asn Tyr Ile Asp His Gly Asp Pro 750 755 760	2607
CAG GTT CGA GGA GCC ACT GCC ATT CTC TGT GGG ACC CTC ATC TGC TCC Gln Val Arg Gly Ala Thr Ala Ile Leu Cys Gly Thr Leu Ile Cys Ser 765 770 775 780	2655
ATC CTC AGC AGG TCC CGC TTC CAC GTG GGA GAT TGG ATG GGC ACC ATT Ile Leu Ser Arg Ser Arg Phe His Val Gly Asp Trp Met Gly Thr Ile 785 790 795	2703
AGA ACC CTC ACA GGA AAT ACA TTT TCT TTG GCG GAT TGC ATT CCT TTG Arg Thr Leu Thr Gly Asn Thr Phe Ser Leu Ala Asp Cys Ile Pro Leu 800 805 810	2751
CTG CCG AAA ACA CTC AAG GAT GAG TCT TCT GTT ACT TGC AAG TTA GCT Leu Arg Lys Thr Leu Lys Asp Glu Ser Ser Val Thr Cys Lys Leu Ala 815 820 825	2799
TGT ACA GCT GTG AGC AAC TGT GTC ATG ACT CTC TGC AGC AGC ACC TAC Cys Thr Ala Val Arg Asn Cys Val Met Ser Leu Cys Ser Ser Ser Tyr 830 835 840	2847
ACT GAG TTA GGA CTG CAG CTG ATC ATC GAT GTG CTG ACT CTG AGG AAC Ser Glu Leu Gly Leu Gln Leu Ile Ile Asp Val Leu Thr Leu Arg Asn 845 850 855 860	2895
AGT TCC TAT TGG CTG GTG AGG ACA GAG CTT CTG GAA ACC CTT GCA GAG Ser Ser Tyr Trp Leu Val Arg Thr Glu Leu Leu Glu Thr Leu Ala Glu 865 870 875	2943
ATT GAC TTC AGG CTG GTG AGC TTT TTG GAG GCA AAA GCA GAA AAC TTA Ile Asp Phe Arg Leu Val Ser Phe Leu Glu Ala Lys Ala Glu Asn Leu 880 885 890	2991
CAC AGA CGG CCT CAT CAT TAT ACA CGG CTT TTA AAA CTG CAA GAA CGA His Arg Gly Ala His His Tyr Thr Gly Leu Leu Lys Leu Gln Glu Arg 895 900 905	3039

FIG.4F

GTG CTC AAT AAT GTT GTC ATC CAT TTG CTT GGA GAT GAA GAC CCC AGG Val Leu Asn Asn Val Val Ile His Leu Leu Gly Asp Glu Asp Pro Arg 910 915 920	3087
GTG CGA CAT GTT GCC GCA GCA TCA CTA ATT AGC CTT GTC CCA AAG CTG Val Arg His Val Ala Ala Ala Ser Leu Ile Arg Leu Val Pro Lys Leu 925 930 935 940	3135
TTT TAT AAA TGT GAC CAA GGA CAA GCT GAT CCA GTC GTC GCC GCA Phe Tyr Lys Cys Asp Gln Gly Gln Ala Asp Pro Val Val Ala Val Ala 945 950 955	3183
AGA GAT CAA AGC AGT GTT TAC CTG AAA CTT CTC ATG CAT GAG ACG CAG Arg Asp Gln Ser Ser Val Tyr Leu Lys Leu Leu Met His Glu Thr Gln 960 965 970	3231
CCT CCA TCT CAT TTC TCC GTC AGC ACA ATA ACC AGA ATA TAT AGA GCC Pro Pro Ser His Phe Ser Val Ser Thr Ile Thr Arg Ile Tyr Arg Gly 975 980 985	3279
TAT AAC CTA CTA CCA AGC ATA ACA GAC GTC ACT ATG GAA AAT AAC CTT Tyr Asn Leu Leu Pro Ser Ile Thr Asp Val Thr Met Glu Asn Asn Leu 990 995 1000	3327
TCA AGA GTT ATT GCA GCA GTT TCT CAT GAA CTA ATC ACA TCA ACC ACC Ser Arg Val Ile Ala Ala Val Ser His Glu Leu Ile Thr Ser Thr Thr 1005 1010 1015 1020	3375
AGA GCA CTC ACA TTT GGA TGC TGT GAA GCT TTG TGT CTT CTT TCC ACT Arg Ala Leu Thr Phe Gly Cys Cys Glu Ala Leu Cys Leu Leu Ser Thr 1025 1030 1035	3423
GCC TTC CCA GTT TGC ATT TGG AGT TTA GGT TGG CAC TGT GCA GTG CCT Ala Phe Pro Val Cys Ile Trp Ser Leu Gly Trp His Cys Gly Val Pro 1040 1045 1050	3471
CCA CTG AGT GCC TCA GAT GAG TCT AGG AAG ACC TGT ACC GTT GGG ATG Pro Leu Ser Ala Ser Asp Glu Ser Arg Lys Ser Cys Thr Val Gly Met 1055 1060 1065	3519

FIG.4G

GCC ACA ATG ATT CTG ACC CTG CTC TCG TCA GCT TGG TTC CCA TTG GAT Ala Thr Met Ile Leu Thr Leu Leu Ser Ser Ala Trp Phe Pro Leu Asp 1070 1075 1080	3567
CTC TCA GCC CAT CAA GAT GCT TTG ATT TTG GCC GGA AAC TTG CTT GCA Leu Ser Ala His Gln Asp Ala Leu Ile Leu Ala Gly Asn Leu Leu Ala 1085 1090 1095 1100	3615
GCC AGT GCT CCC AAA TCT CTG AGA AGT TCA TGG GCC TCT GAA GAA GAA Ala Ser Ala Pro Lys Ser Leu Arg Ser Ser Trp Ala Ser Glu Glu Glu 1105 1110 1115	3663
GCC AAC CCA GCA GCC ACC AAG CAA GAG GAC GTC TGG CCA GCC CTG GGG Ala Asn Pro Ala Ala Thr Lys Gln Glu Val Trp Pro Ala Leu Gly 1120 1125 1130	3711
GAC CGG CCC CTG GTG CCC ATG GTG GAG CAG CTC TTC TCT CAC CTG CTG Asp Arg Ala Leu Val Pro Met Val Glu Gln Leu Phe Ser His Leu Leu 1135 1140 1145	3759
AAG CTG ATT AAC ATT TGT GCC CAC GTC CTC GAT GAC CTG GCT CCT GGA Lys Val Ile Asn Ile Cys Ala His Val Leu Asp Asp Val Ala Pro Gly 1150 1155 1160	3807
CCC GCA ATA AAG GCA GCC TTG CCT TCT CTA ACA AAC CCC CCT TCT CTA Pro Ala Ile Lys Ala Ala Leu Pro Ser Leu Thr Asn Pro Pro Ser Leu 1165 1170 1175 1180	3855
AGT CCC ATC CGA CGA AAG GGG AAG GAG AAA GAA CCA GGA GAA CAA GCA Ser Pro Ile Arg Arg Lys Gly Lys Lys Glu Pro Gly Glu Gln Ala 1185 1190 1195	3903
TCT GTA CCG TTG ACT CCC AAG AAA GGC ACT GAG GCC ACT GCA GCT TCT Ser Val Pro Leu Ser Pro Lys Lys Gly Ser Glu Ala Ser Ala Ala Ser 1200 1205 1210	3951
AGA CAA TCT GAT ACC TCA CGT CCT GTT ACA ACA ACT AAA TCC TCA TCA Arg Gln Ser Asp Thr Ser Gly Pro Val Thr Thr Ser Lys Ser Ser Ser 1215 1220 1225	3999

FIG.4H

CTG GGG AGT TTC TAT CAT CTT CCT TCA TAC CTC AGA CTG CAT GAT GTC Leu Gly Ser Phe Tyr His Leu Pro Ser Tyr Leu Arg Leu His Asp Val 1230 1235 1240	4047
CTG AAA GCT ACA CAC GCT AAC TAC AAG GTC ACG CTG GAT CTT CAG AAC Leu Lys Ala Thr His Ala Asn Tyr Lys Val Thr Leu Asp Leu Gln Asn 1245 1250 1255 1260	4095
ACC ACG GAA AAG TTT GGA GGG TTT CTC CGC TCA GCC TTG GAT GTT CTT Ser Thr Glu Lys Phe Gly Gly Phe Leu Arg Ser Ala Leu Asp Val Leu 1265 1270 1275	4143
TCT CAG ATA CTA GAG CTG GCC ACA CTG CAG GAC ATT CGG AAC TGT GTT Ser Gln Ile Leu Glu Leu Ala Thr Leu Gln Asp Ile Gly Lys Cys Val 1280 1285 1290	4191
GAA GAG ATC CTA GGA TAC CTG AAA TCC TGC TTT AGT CGA GAA CCA ATG Glu Glu Ile Leu Gly Tyr Leu Lys Ser Cys Phe Ser Arg Glu Pro Met 1295 1300 1305	4239
ATG GCA ACT GTT TGT GTT CAA CAA TTG TTG AAG ACT CTC TTT GGC ACA Met Ala Thr Val Cys Val Gln Gln Leu Leu Lys Thr Leu Phe Gly Thr 1310 1315 1320	4287
AAC TTG GCC TCC CAG TTT GAT GGC TTA TCT TCC AAC CCC ACC AAG TCA Asn Leu Ala Ser Gln Phe Asp Gly Leu Ser Ser Asn Pro Ser Lys Ser 1325 1330 1335 1340	4335
CAA GGC CGA CGA CAG CGC CTT GGC TCC TCC AGT GTG AGG CCA CGC TTG Gln Gly Arg Ala Gln Arg Leu Gly Ser Ser Ser Val Arg Pro Gly Leu 1345 1350 1355	4383
TAC CAC TAC TGC TTC ATG GCC CGG TAC ACC CAC TTC ACC CAG CCC CTC Tyr His Tyr Cys Phe Met Ala Pro Tyr Thr His Phe Thr Gln Ala Leu 1360 1365 1370	4431
GCT GAC GCC AGC CTG AGG AAC ATG GTG CAG GCG GAG CAG GAC AAC GAC Ala Asp Ala Ser Leu Arg Asn Met Val Gln Ala Glu Gln Glu Asn Asp 1375 1380 1385	4479

FIG.41

ACC TCG GGA TGG TTT GAT GTC CTC CAG AAA GTC TCT ACC CAG TTG AAC	4527
Thr Ser Gly Trp Phe Asp Val Leu Gln Lys Val Ser Thr Gln Leu Lys	
1390 1395 1400	
ACA AAC CTC ACC AGT GTC ACA AAG AAC CGT GCA GAT AAG AAT GCT ATT	4575
Thr Asn Leu Thr Ser Val Thr Lys Asn Arg Ala Asp Lys Asn Ala Ile	
1405 1410 1415 1420	
CAT AAT CAC ATT CGT TTG TTT GAA CCT CTT GTT ATA AAA GCT TTA AAA	4623
His Asn His Ile Arg Leu Phe Glu Pro Leu Val Ile Lys Ala Leu Lys	
1425 1430 1435	
CAG TAC ACC ACT ACA ACA TGT GTG CAG TTA CAG AAG CAC GTT TTA GAT	4671
Gln Tyr Thr Thr Cys Val Gln Leu Gln Lys Gln Val Leu Asp	
1440 1445 1450	
TTG CTG GCG CAG CTG GTT CAG TTA CGG GTT AAT TAC TGT CTT CTG GAT	4719
Leu Leu Ala Gln Leu Val Gln Leu Arg Val Asn Tyr Cys Leu Leu Asp	
1455 1460 1465	
TCA GAT CAG GTG TTT ATT GGC TTT GTC TTG AAA CAG TTT GAA TAC ATT	4767
Ser Asp Gln Val Phe Ile Gly Phe Val Leu Lys Gln Phe Glu Tyr Ile	
1470 1475 1480	
GAA GTG GGC CAG TTC AGG GAA TCA GAG GCA ATC ATT CCA AAC ATC TTT	4815
Glu Val Gly Gln Phe Arg Glu Ser Glu Ala Ile Ile Pro Asn Ile Phe	
1485 1490 1495 1500	
TTC TTC TTG GTC TTA CTA TCT TAT GAA CGC TAT CAT TCA AAA CAG ATC	4863
Phe Phe Leu Val Leu Leu Ser Tyr Glu Arg Tyr His Ser Lys Gln Ile	
1505 1510 1515	
ATT GGA ATT CCT AAA ATC ATT CAG CTC TGT GAT GGC ATC ATG GCC AGT	4911
Ile Gly Ile Pro Lys Ile Ile Gln Leu Cys Asp Gly Ile Met Ala Ser	
1520 1525 1530	
GGA AGG AAG GCT GTG ACA CAT GCC ATA CGG GCT CTG CAG CCC ATA GTC	4959
Gly Arg Lys Ala Val Thr His Ala Ile Pro Ala Leu Gln Pro Ile Val	
1535 1540 1545	

FIG.4J

CAC GAC CTC TTT GTA TTA AGA CGA ACA AAT AAA GCT GAT CCA GGA AAA		5007
His Asp Leu Phe Val Leu Arg Gly Thr Asn Lys Ala Asp Ala Gly Lys		
1550	1555	1560
GAG CTT GAA ACC CAA AAA GAG GTG GTG GTG TCA ATG TTA CTG AGA CTC		5055
Glu Leu Glu Thr Gln Lys Glu Val Val Val Ser Met Leu Leu Arg Leu		
1565	1570	1575
1580		
ATC CAG TAC CAT CAG GTG TTG GAG ATG TTC ATT CTT GTC CTC CAG CAC		5103
Ile Gln Tyr His Gln Val Leu Glu Met Phe Ile Leu Val Leu Gln Gln		
1585	1590	1595
TGC CAC AAC GAG AAT GAA GAC AAG TCG AAG CGA CTG TCT CGA CAG ATA		5151
Cys His Lys Glu Asn Glu Asp Lys Trp Lys Arg Leu Ser Arg Gln Ile		
1600	1605	1610
GCT GAC ATC ATC CTC CCA ATG TTA GCC AAA CAG CAG ATG CAC ATT GAC		5199
Ala Asp Ile Ile Leu Pro Met Leu Ala Lys Gln Gln Met His Ile Asp		
1615	1620	1625
TCT CAT GAA GCC CTT CGA GTG TTA AAT ACA TTA TTT GAG ATT TTG GCC		5247
Ser His Glu Ala Leu Gly Val Leu Asn Thr Leu Phe Glu Ile Leu Ala		
1630	1635	1640
CCT TCC TCC CTC CGT CCG GTA GAC ATG CTT TTA CCG ACT ATG TTC GTC		5295
Pro Ser Ser Leu Arg Pro Val Asp Met Leu Leu Arg Ser Met Phe Val		
1645	1650	1655
1660		
ACT CCA AAC ACA ATG GCG TCC GTG AGC ACT GTT CAA CTG TGG ATA TCG		5343
Thr Pro Asn Thr Met Ala Ser Val Ser Thr Val Gln Leu Trp Ile Ser		
1665	1670	1675
GGA ATT CTG GCC ATT TTG AGG CTT CTG ATT TCC CAG TCA ACT GAA GAT		5391
Gly Ile Leu Ala Ile Leu Arg Val Leu Ile Ser Gln Ser Thr Glu Asp		
1680	1685	1690
ATT GTT CTT TCT CGT ATT CAG GAG CTC TCC TTC TCT CCG TAT TTA ATC		5439
Ile Val Leu Ser Arg Ile Gln Glu Leu Ser Phe Ser Pro Tyr Leu Ile		
1695	1700	1705

FIG.4K

TCC TGT ACA GTA ATT AAT AGG TTA AGA GAT GGG GAC AGT ACT TCA ACC	5487
Ser Cys Thr Val Ile Asn Arg Leu Arg Asp Gly Asp Ser Thr Ser Thr	
1710 1715 1720	
CTA GAA GAA CAC AGT GAA GGG AAA CAA ATA AAG AAT TTG CCA GAA GAA	5535
Leu Glu Glu His Ser Glu Gly Lys Gln Ile Lys Asn Leu Pro Glu Glu	
1725 1730 1735 1740	
ACA TTT TCA AGG TTT CTA TTA CAA CTG GTT GCT ATT CTT TTA GAA GAC	5583
Thr Phe Ser Arg Phe Leu Leu Gln Leu Val Gly Ile Leu Leu Glu Asp	
1745 1750 1755	
ATT GTT ACA AAA CAG CTG AAG GTG GAA ATG AGT GAG CAG CAA CAT ACT	5631
Ile Val Thr Lys Gln Leu Lys Val Glu Met Ser Glu Gln Gln His Thr	
1760 1765 1770	
TTC TAT TCC CAG GAA CTA GGC ACA CTG CTA ATG TGT CTC ATC CAC ATC	5679
Phe Tyr Cys Gln Glu Leu Gly Thr Leu Leu Met Cys Leu Ile His Ile	
1775 1780 1785	
TTC AAG TCT GGA ATG TTC CCG AGA ATC ACA GCA GCT CCC ACT AGG CTC	5727
Phe Lys Ser Gly Met Phe Arg Arg Ile Thr Ala Ala Ala Thr Arg Leu	
1790 1795 1800	
TTC CGC AGT GAT GGC TGT GGC GGC AGT TTC TAC ACC CTG GAC AGC TTG	5775
Phe Arg Ser Asp Gly Cys Gly Ser Phe Tyr Thr Leu Asp Ser Leu	
1805 1810 1815 1820	
AAC TTC CGG GCT CGT TCC ATG ATC ACC ACC CAC CCC CCC CTG GTC CTG	5823
Asn Leu Arg Ala Arg Ser Met Ile Thr Thr His Pro Ala Leu Val Leu	
1825 1830 1835	
CTC TGG TGT CAG ATA CTG CTG CTT GTC AAC CAC ACC GAC TAC CGC TCG	5871
Leu Trp Cys Gln Ile Leu Leu Leu Val Asn His Thr Asp Tyr Arg Trp	
1840 1845 1850	

FIG.4L

TGG GCA GAA GTG CAG CAG ACC CCG AAA AGA CAC AGT CTG TCC AGC ACA Trp Ala Glu Val Gln Gln Thr Pro Lys Arg His Ser Leu Ser Ser Thr 1855 1860 1865	5919
AAG TTA CTT AGT CCC CAG ATG TCT GGA GAA GAG GAC GAT TCT GAC TTG Lys Leu Leu Ser Pro Gln Met Ser Gly Glu Glu Asp Ser Asp Leu 1870 1875 1880	5967
GCA GCC AAA CTT GGA ATG TGC AAT AGA GAA ATA GTC CGA AGA GGG CCT Ala Ala Lys Leu Gly Met Cys Asn Arg Glu Ile Val Arg Arg Gly Ala 1885 1890 1895 1900	6015
CTC ATT CTC TTC TGT CAT TAT GTC TGT CAG AAC CTC CAT GAC TCC GAG Leu Ile Leu Phe Cys Asp Tyr Val Cys Gln Asn Leu His Asp Ser Glu 1905 1910 1915	6063
CAC TTA ACC TGG CTC ATT GTC AAT CAC ATT CAA GAT CTG ATC AGC CTT His Leu Thr Trp Leu Ile Val Asn His Ile Gln Asp Leu Ile Ser Leu 1920 1925 1930	6111
TCC CAC GAG CCT CCA GTC CAG GAC TTC ATC ACT GCC GTT CAT CGG AAC Ser His Glu Pro Pro Val Gln Asp Phe Ile Ser Ala Val His Arg Asn 1935 1940 1945	6159
TCT GCT GCC AGC GGC CTG TTC ATC CAG GCA ATT CAG TCT CGT TGT GAA Ser Ala Ala Ser Gly Leu Phe Ile Gln Ala Ile Gln Ser Arg Cys Glu 1950 1955 1960	6207
AAC CTT TCA ACT CCA ACC ATG CTG AAG AAA ACT CTT CAG TGC TTG GAG Asn Leu Ser Thr Pro Thr Met Leu Lys Lys Thr Leu Gln Cys Leu Glu 1965 1970 1975 1980	6255
GGG ATC CAT CTC AGC CAG TCG GGA GCT GTG CTC ACC CTG TAT GTG GAC Gly Ile His Leu Ser Gln Ser Gly Ala Val Leu Thr Leu Tyr Val Asp 1985 1990 1995	6303
AGG CTT CTG TGC ACC CCT TTC CGT GTG CTC GCT CGC ATG GTC GAC ATC Arg Leu Leu Cys Thr Pro Phe Arg Val Leu Ala Arg Met Val Asp Ile 2000 2005 2010	6351

FIG.4M

CTT GCT TGT CCC CGG CTA GAA ATG CTT CTG GCT GCA AAT TTA CAG AGC Leu Ala Cys Arg Arg Val Glu Met Leu Leu Ala Ala Asn Leu Gln Ser 2015 2020 2025	6399
AGC ATG GCC CAG TTG CCA ATG GAA GAA CTC AAC AGA ATC CAG GAA TAC Ser Met Ala Gln Leu Pro Met Glu Glu Leu Asn Arg Ile Gln Glu Tyr 2030 2035 2040	6447
CTT CAG AGC AGC GGG CTC GCT CAG AGA CAC CAA AGG CTC TAT TCC CTG Leu Gln Ser Ser Gly Leu Ala Gln Arg His Gln Arg Leu Tyr Ser Leu 2045 2050 2055 2060	6495
CTG GAC AGG TTT CGT CTC TCC ACC ATG CAA GAC TCA CTT ACT CCC TCT Leu Asp Arg Phe Arg Leu Ser Thr Met Gln Asp Ser Leu Ser Pro Ser 2065 2070 2075	6543
CCT CCA GTC TCT TCC CAC CCG CTG GAC GGG GAT GGG CAC GTG TCA CTG Pro Pro Val Ser Ser His Pro Leu Asp Gly Asp Gly His Val Ser Leu 2080 2085 2090	6591
GAA ACA GTG AGT CCG GAC AAA GAC TGG TAC GTT CAT CTT GTC AAA TCC Glu Thr Val Ser Pro Asp Lys Asp Trp Tyr Val His Leu Val Lys Ser 2095 2100 2105	6639
CAG TGT TGG ACC AGG TCA GAT TCT GCA CTG CTG GAA GGT GCA GAG CTG Gln Cys Trp Thr Arg Ser Asp Ser Ala Leu Leu Glu Gly Ala Glu Leu 2110 2115 2120	6687
GTG AAT CGG ATT CCT GCT GAA GAT ATG AAT GCC TTC ATG ATG AAC TCC Val Asn Arg Ile Pro Ala Glu Asp Met Asn Ala Phe Met Met Asn Ser 2125 2130 2135 2140	6735
GAG TTC AAC CTA ACC CTG CTA GCT CCA TGC TTA AGC CTA GGG ATG AGT Glu Phe Asn Leu Ser Leu Leu Ala Pro Cys Leu Ser Leu Gly Met Ser 2145 2150 2155	6783
GAA ATT TCT GGT GCC CAG AAG AGT GCC CTT TTT GAA GCA GCC CGT GAG Glu Ile Ser Gly Gly Gln Lys Ser Ala Leu Phe Glu Ala Ala Arg Glu 2160 2165 2170	6831

FIG.4N

GTG ACT CTG GCC CCT GTG ACC GGC ACC GTG CAG CAG CTC CCT GTC Val Thr Leu Ala Arg Val Ser Gly Thr Val Gln Gln Leu Pro Ala Val 2175 2180 2185	6879
CAT CAT GTC TTC CAG CCC GAG CTG CCT GCA GAG CGG GCG GCC TAC TCG His His Val Phe Gln Pro Glu Leu Pro Ala Glu Pro Ala Ala Tyr Trp 2190 2195 2200	6927
AGC AAG TTG AAT GAT CTG TTT GGG GAT GCT GCA CTG TAT CAG TCC CTG Ser Lys Leu Asn Asp Leu Phe Gly Asp Ala Ala Leu Tyr Gln Ser Leu 2205 2210 2215 2220	6975
CCC ACT CTG GCC CGG GCC CTG GCA CAG TAC CTG GTG GTG GTC TCC AAA Pro Thr Leu Ala Arg Ala Leu Ala Gln Tyr Leu Val Val Val Ser Lys 2225 2230 2235	7023
CTG CCC AGT CAT TTG CAC CTT CCT CCT GAG AAA GAG AAG GAC ATT GTG Leu Pro Ser His Leu His Leu Pro Pro Glu Lys Glu Lys Asp Ile Val 2240 2245 2250	7071
AAA TTC GTG GTG GCA ACC CTT GAG GCC CTG TCC TGG CAT TTG ATC CAT Lys Phe Val Val Ala Thr Leu Glu Ala Leu Ser Trp His Leu Ile His 2255 2260 2265	7119
GAG CAG ATC CCG CTG AGT CTG GAT CTC CAG GCA CGG CTG GAC TGC TGC Glu Gln Ile Pro Leu Ser Leu Asp Leu Gln Ala Gly Leu Asp Cys Cys 2270 2275 2280	7167
TGC CTG GCC CTG CAG CTG CCT CGC CTC TGG AGC GTG GTC TCC TCC ACA Cys Leu Ala Leu Gln Leu Pro Gly Leu Trp Ser Val Val Ser Ser Thr 2285 2290 2295 2300	7215
GAG TTT GTG ACC CAC GGC TGC TCC CTC ATC TAC TGT GTG CAC TTC ATC Glu Phe Val Thr His Ala Cys Ser Leu Ile Tyr Cys Val His Phe Ile 2305 2310 2315	7263
CTG GAG GCC GTT GCA GTG CAG CCT GGA GAG CAG CTT CTT AGT CCA GAA Leu Glu Ala Val Ala Val Gln Pro Gly Glu Gln Leu Leu Ser Pro Glu 2320 2325 2330	7311

FIG.40

AGA AGG ACA AAT ACC CCA AAA GCC ATC AGC GAG GAG GAG GAA GTA Arg Arg Thr Asn Thr Pro Lys Ala Ile Ser Glu Glu Glu Glu Val 2335 2340 2345	7359
CAT CCA AAC ACA CAC AAT CCT AAG TAT ATC ACT GCA GCC TGT GAG ATG Asp Pro Asn Thr Gln Asn Pro Lys Tyr Ile Thr Ala Ala Cys Glu Met 2350 2355 2360	7407
GTC GCA GAA ATC GTG GAG TCT CTG CAG TCG GTC TTG CCC TTG CGT CAT Val Ala Glu Met Val Glu Ser Leu Gln Ser Val Leu Ala Leu Gly His 2365 2370 2375 2380	7455
AAA AGG AAT AGC GCC GTG CCC CGG TTT CTC ACG CCA TTG CTC AGG AAC Lys Arg Asn Ser Gly Val Pro Ala Phe Leu Thr Pro Leu Leu Arg Asn 2385 2390 2395	7503
ATC ATC ATC AGC CTG GCC CGC CTG CCC CTT GTC AAC AGC TAC ACA CGT Ile Ile Ile Ser Leu Ala Arg Leu Pro Leu Val Asn Ser Tyr Thr Arg 2400 2405 2410	7551
GTC CCC CCA CTG GTG TCG AAG CTT GGA TGG TCA CCC AAA CCG GGA GGG Val Pro Pro Leu Val Trp Lys Leu Gly Trp Ser Pro Lys Pro Gly Gly 2415 2420 2425	7599
GAT TTT GCC ACA GCA TTC CCT GAG ATC CCC GTG GAG TTC CTC CAG GAA Asp Phe Gly Thr Ala Phe Pro Glu Ile Pro Val Glu Phe Leu Gln Glu 2430 2435 2440	7647
AAG GAA GTC TTT AAG GAG TTC ATC TAC CGC ATC AAC ACA CTA CGC TGG Lys Glu Val Phe Lys Glu Phe Ile Tyr Arg Ile Asn Thr Leu Gly Trp 2445 2450 2455 2460	7695
ACC AGT CGT ACT CAG TTT GAA GAA ACT TGG GCC ACC CTC CTT GGT GTC Thr Ser Arg Thr Gln Phe Glu Glu Thr Trp Ala Thr Leu Leu Gly Val 2465 2470 2475	7743
CTG GTG ACC CAG CCC CTC GTG ATG GAG CAG GAG GAG AGC CCA CCA GAA Leu Val Thr Gln Pro Leu Val Met Glu Gln Glu Glu Ser Pro Pro Glu 2480 2485 2490	7791

FIG.4P

GAA GAC ACA GAG AGG ACC CAG ATC AAC GTC CTG GCC GTG CAG GCC ATC Glu Asp Thr Glu Arg Thr Gln Ile Asn Val Leu Ala Val Gln Ala Ile 2495 2500 2505	7839
ACC TCA CTG GTG CTC AGT GCA ATG ACT GTG CCT CTG GCC GGC AAC CCA Thr Ser Leu Val Leu Ser Ala Met Thr Val Pro Val Ala Gly Asn Pro 2510 2515 2520	7887
GCT GTA AGC TGC TTG GAG CAG CAG CCC CGG AAC AAG CCT CTG AAA GCT Ala Val Ser Cys Leu Glu Gln Gln Pro Arg Asn Lys Pro Leu Lys Ala 2525 2530 2535 2540	7935
CTC GAC ACC AGG TTT GGG AGC AAG CTG ACC ATT ATC AGA GGG ATT CTG Leu Asp Thr Arg Phe Gly Arg Lys Leu Ser Ile Ile Arg Gly Ile Val 2545 2550 2555	7983
GAG CAA GAG ATT CAA GCA ATG GTT TCA AAG AGA GAG AAT ATT GCC ACC Glu Gln Glu Ile Gln Ala Met Val Ser Lys Arg Glu Asn Ile Ala Thr 2560 2565 2570	8031
CAT CAT TTA TAT CAG GCA TGG CAT CCT GTC CCT TCT CTG TCT CCG GCT His His Leu Tyr Gln Ala Trp Asp Pro Val Pro Ser Leu Ser Pro Ala 2575 2580 2585	8079
ACT ACA GGT GCC CTC ATC AGC CAC GAG AAG CTG CTG CTA CAG ATC AAC Thr Thr Gly Ala Leu Ile Ser His Glu Lys Leu Leu Glu Ile Asn 2590 2595 2600	8127
CCC GAG CGG GAG CTG GGG AGC ATG AGC TAC AAA CTC GGC CAG GTG TCC Pro Glu Arg Glu Leu Gly Ser Met Ser Tyr Lys Leu Gly Gln Val Ser 2605 2610 2615 2620	8175
ATA CAC TCC GTG TCG CTG GGG AAC AGC ATC ACA CCC CTG ACC GAG GAG Ile His Ser Val Trp Leu Gly Asn Ser Ile Thr Pro Leu Arg Glu Glu 2625 2630 2635	8223
GAA TGG GAC GAG GAA GAG GAG GAG GAG GCC GAC GCC CCT GCA CCT TCG Glu Trp Asp Glu Glu Glu Glu Glu Ala Asp Ala Pro Ala Pro Ser 2640 2645 2650	8271

FIG.4Q

TCA CCA CCC ACG TCT CCA GTC AAC TCC AGG AAA CAC CCG GCT GGA GTT	8319
Ser Pro Pro Thr Ser Pro Val Asn Ser Arg Lys His Arg Ala Gly Val	
2655 2660 2665	
GAC ATC CAC TCC TGT TCG CAG TTT TTG CTT GAG TTG TAC AGC CGC TGG	8367
Asp Ile His Ser Cys Ser Gln Phe Leu Leu Glu Leu Tyr Ser Arg Trp	
2670 2675 2680	
ATC CTG CCC TCC ACC TCA GCC ACC ACC CCG GCC ATC CTG ATC ACT	8415
Ile Leu Pro Ser Ser Ser Ala Arg Arg Thr Pro Ala Ile Leu Ile Ser	
2685 2690 2695 2700	
GAG GTG GTC AGA TCC CTT CTA GTC GTC TCA GAC TTG TTC ACC CAG CGC	8463
Glu Val Val Arg Ser Leu Leu Val Val Ser Asp Leu Phe Thr Glu Arg	
2705 2710 2715	
AAC CAG TTT GAG CTG ATG TAT GTG ACG CTG ACA GAA CTG CGA AGG GTG	8511
Asn Gln Phe Glu Leu Met Tyr Val Thr Leu Thr Glu Leu Arg Arg Val	
2720 2725 2730	
CAC CCT TCA GAA GAC GAG ATC CTC GCT CAG TAC CTG GTG CCT GCC ACC	8559
His Pro Ser Glu Asp Glu Ile Leu Ala Gln Tyr Leu Val Pro Ala Thr	
2735 2740 2745	
TGC AAG GCA GCT GCC GTC CTT GGG ATG GAC AAG GCC GTG GCG GAG CCT	8607
Cys Lys Ala Ala Ala Val Leu Gly Met Asp Lys Ala Val Ala Glu Pro	
2750 2755 2760	
GTC AGC CGC CTG CTG GAG ACG ACG CTC AGC AGC AGC CAC CTG CCC AGC	8655
Val Ser Arg Leu Leu Glu Ser Thr Leu Arg Ser Ser His Leu Pro Ser	
2765 2770 2775 2780	
AGG GTT GGA GCC CTG CAC CGC ATC CTC TAT GTG CTG GAG TGC GAC CTG	8703
Arg Val Glu Ala Leu His Glu Ile Leu Tyr Val Leu Glu Cys Asp Leu	
2785 2790 2795	
CTG GAC GAC ACT GCC AAG CAG CTC ATC CCG GTC ATC AGC GAC TAT CTC	8751
Leu Asp Asp Thr Ala Lys Gln Leu Ile Pro Val Ile Ser Asp Tyr Leu	
2800 2805 2810	

FIG.4R

CTC TCC AAC CTG AAA CGG ATC GCC CAC TGC GTG AAC ATT CAC AGC CAG Leu Ser Asn Leu Lys Gly Ile Ala His Cys Val Asn Ile His Ser Gln 2815 2820 2825	8799
CAG CAC GTA CTG GTC ATC TGT GCC ACT GCG TTT TAC CTC ATT GAG AAC Gln His Val Leu Val Met Cys Ala Thr Ala Phe Tyr Leu Ile Glu Asn 2830 2835 2840	8847
TAT CCT CTG GAC GTA GGG CCG GAA TTT TCA GCA TCA ATA ATA CAG ATG Tyr Pro Leu Asp Val Gly Pro Glu Phe Ser Ala Ser Ile Ile Gln Met 2845 2850 2855 2860	8895
TGT GGG GTG ATG CTG TCT GGA ACT GAG GAG TCC ACC CCC TCC ATC ATT Cys Gly Val Met Leu Ser Gly Ser Glu Glu Ser Thr Pro Ser Ile Ile 2865 2870 2875	8943
TAC CAC TGT GCC CTC AGA GGC CTG GAC CCG CTC CTG CTC TCT GAC CAG Tyr His Cys Ala Leu Arg Gly Leu Glu Arg Leu Leu Ser Glu Gln 2880 2885 2890	8991
CTC TCC CGC CTG GAT GCA GAA TCG CTG GTC AAG CTG AGT GTG GAC AGA Leu Ser Arg Leu Asp Ala Glu Ser Leu Val Lys Leu Ser Val Asp Arg 2895 2900 2905	9039
GTG AAC GTG CAC AGC CCG CAC CGG GCC ATG GCG GCT CTG GGC CTG ATG Val Asn Val His Ser Pro His Arg Ala Met Ala Ala Leu Gly Leu Met 2910 2915 2920	9087
CTC ACC TGC ATG TAC ACA CGA AAC GAC AAA GTC AGT CCC GGT AGA ACT Leu Thr Cys Met Tyr Thr Gly Lys Glu Lys Val Ser Pro Gly Arg Thr 2925 2930 2935 2940	9135
TCA GAC CCT AAT CCT GCA GCC CCC GAC AGC GAG TCA GTG ATT GTT GCT Ser Asp Pro Asn Pro Ala Ala Pro Asp Ser Glu Ser Val Ile Val Ala 2945 2950 2955	9183
ATG GAG CGG GTA TCT GTT CTT TTT GAT AGG ATC AGG AAA GCC TTT CCT Met Glu Arg Val Ser Val Leu Phe Asp Arg Ile Arg Lys Gly Phe Pro 2960 2965 2970	9231

FIG.4S

TGT GAA GCC AGA GTG GTG GCC AGG ATC CTG CCC CAG TTT CTA GAC GAC Cys Glu Ala Arg Val Val Ala Arg Ile Leu Pro Gln Phe Leu Asp Asp 2975 2980 2985	9279
TTC TTC CCA CCC CAG GAC ATC ATG AAC AAA GTC ATC CGA GAG TTT CTC Phe Phe Pro Pro Gln Asp Ile Met Asn Lys Val Ile Gly Glu Phe Leu 2990 2995 3000	9327
TCC AAC CAG CAG CCA TAC CCC CAG TTC ATG GCC ACC GTG GTG TAT AAG Ser Asn Gln Gln Pro Tyr Pro Gln Phe Met Ala Thr Val Val Tyr Lys 3005 3010 3015 3020	9375
GTG TTT CAG ACT CTG CAC AGC ACC GGG CAG TCG TCC ATG GTC CGG GAC Val Phe Gln Thr Leu His Ser Thr Gly Gln Ser Ser Met Val Arg Asp 3025 3030 3035	9423
TGG GTC ATG CTG TCC CTC TCC AAC TTC ACG CAG AGG GCC CCG GTC GCC Trp Val Met Leu Ser Leu Ser Asn Phe Thr Gln Arg Ala Pro Val Ala 3040 3045 3050	9471
ATG GCC ACC TGG AGC CTC TCC TGC TTC TTT GTC AGC GCG TCC ACC AGC Met Ala Thr Trp Ser Leu Ser Cys Phe Phe Val Ser Ala Ser Thr Ser 3055 3060 3065	9519
CCG TGG GTC GCG GCG ATC CTC CCA CAT GTC ATC AGC AGG ATG GGC AAG Pro Trp Val Ala Ala Ile Leu Pro His Val Ile Ser Arg Met Gly Lys 3070 3075 3080	9567
CTG GAG CAG GTG GAC GTG AAC CTT TTC TGC CTG GTC GCC ACA GAC TTC Leu Glu Gln Val Asp Val Asn Leu Phe Cys Leu Val Ala Thr Asp Phe 3085 3090 3095 3100	9615
TAC AGA CAC CAG ATA GAG GAG GAC CTC GAC CGC AGG GCC TTC CAG TCT Tyr Arg His Gln Ile Glu Glu Glu Leu Asp Arg Arg Ala Phe Gln Ser 3105 3110 3115	9663

FIG.4T

GTG CTT GAG GTG GTT GCA GCC CCA GGA AGC CCA TAT CAC CGG CTG CTG Val Leu Glu Val Val Ala Ala Pro Gly Ser Pro Tyr His Arg Leu Leu 3120 3125 3130	9711
ACT TGT TTA CGA AAT GTC CAC AAG GTC ACC ACC TGC T GAGGCCATG Thr Cys Leu Arg Asn Val His Lys Val Thr Thr Cys 3135 3140	9758
GTGGGAGAGA CTGTGAGGCC GCAGCTGGGG CGGGAGCCTT TGGAACTCTG TGGCCCTTG CCCTGCCTCC ACGAGGCCAG CTTGGTCCCT ATGGGCTTCC GCACATGCCG CGGGGGCCCA GGCAACGTGC GTGTCTCTGC CATGTGCCAG AAGTGCCTT TGTGGCAGTC GCCAGCCAG GAGTGTCTGC AGTCCTGGTG GGGCTGAGCC TGAGGCCCTC CAGAAAGCAG GAGCAGCTGT GCTGCACCCC ATGTGGGTGA CCAGGTCCTT TCTCCTGATA GTCACCTGCT GGTGTTGCC AGGTTGCAGC TGCTCTTGCA TCTGGCCAG AAGTCCTCCC TCCGTGAGGC TGGCTGTTGC CCCTCTGCT GTCTGCAGT AGAAGGTGCC GTGAGCAGGC TTTGGGAACA CTGGCCTGG TCTCCCTGGT GGGGTGTGCA TGCCACGCC CGTGTCTGGA TGCACAGATG CCATGGCCTG TGCTGGCCA GTGGCTGGGG GTGCTAGACA CCGGGCACCA TTCTCCCTTC TCTCTTTCT TCTCAGGATT TAAAATTAA TTATATCACT AAAGAGATTA ATTTAACGT AAAAAAAA AAAAAAA	9818 9878 9938 9998 10058 10118 10178 10238 10298 10358 10366

FIG.4U

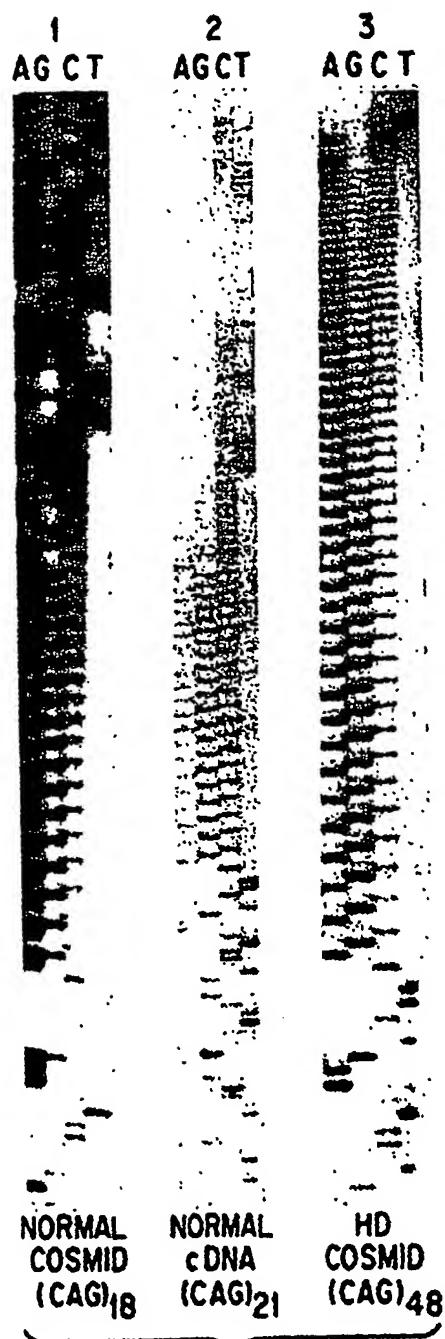


FIG. 5

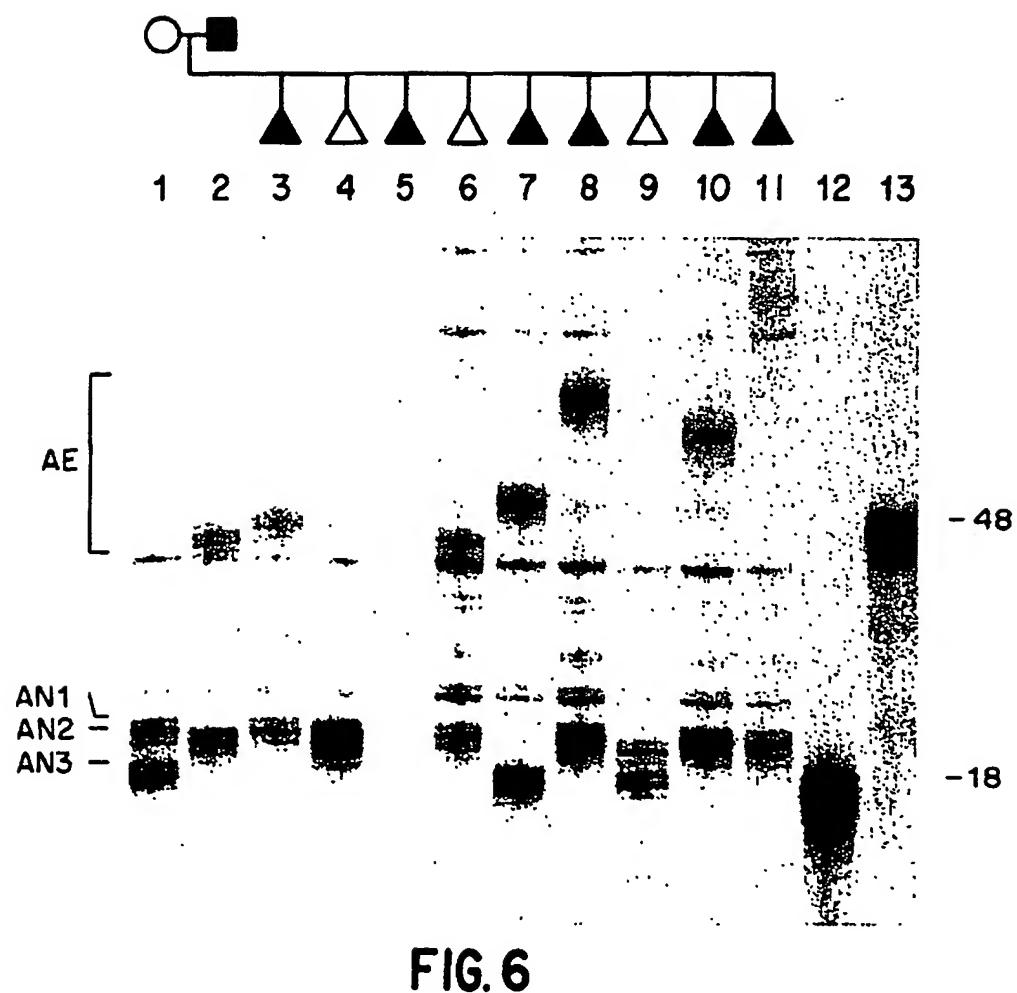
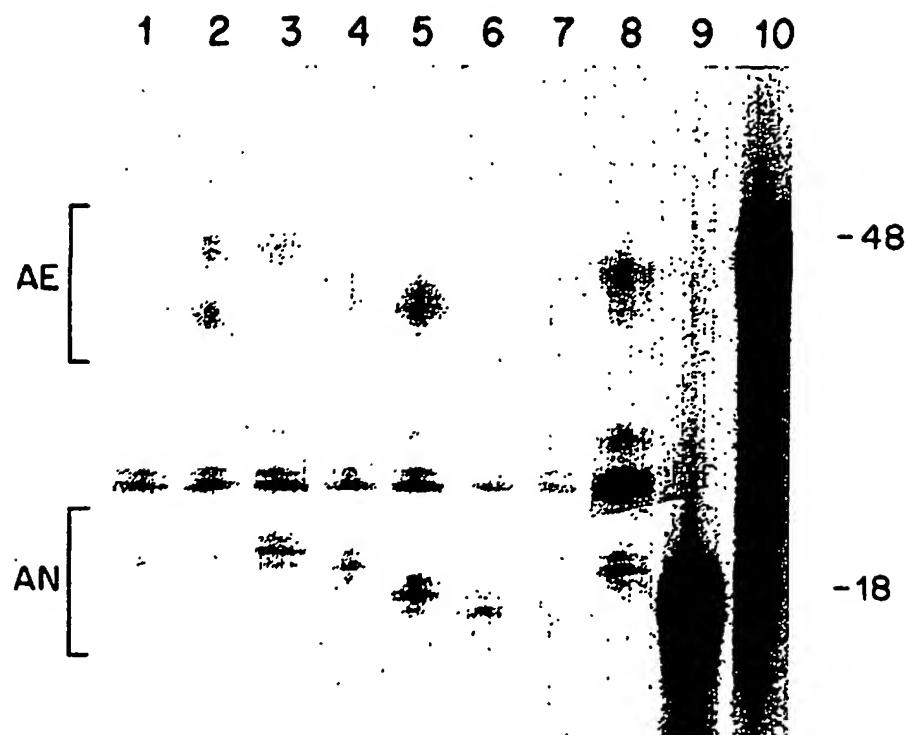


FIG. 6



FIG. 7



**FIG. 8**



FIG. 9

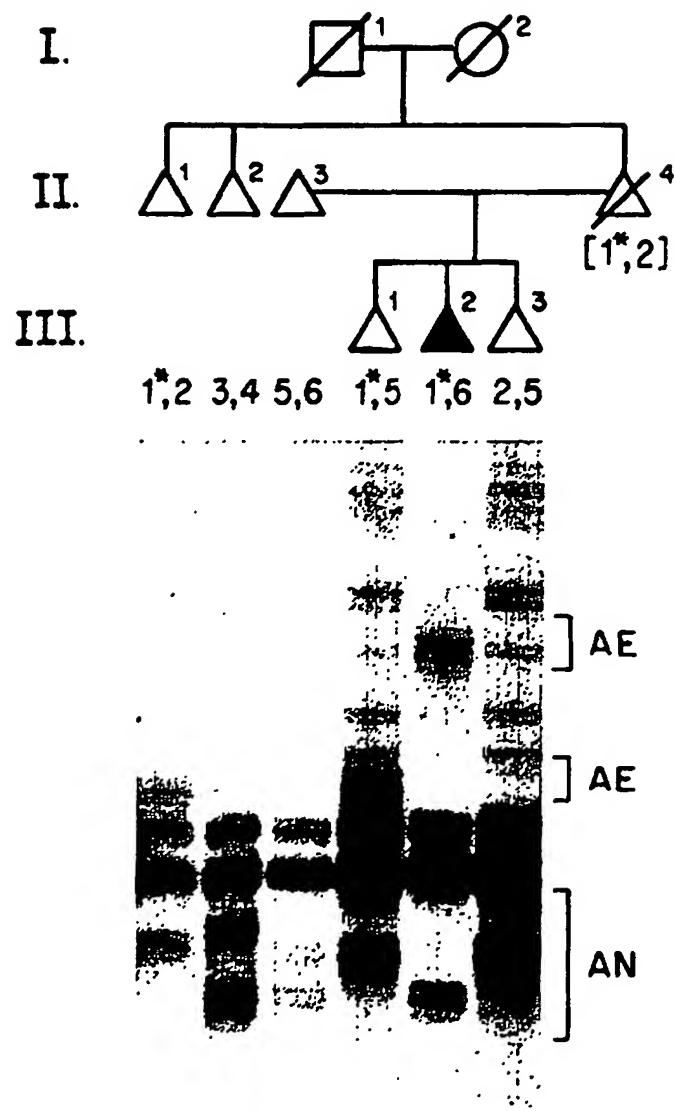


FIG. 10

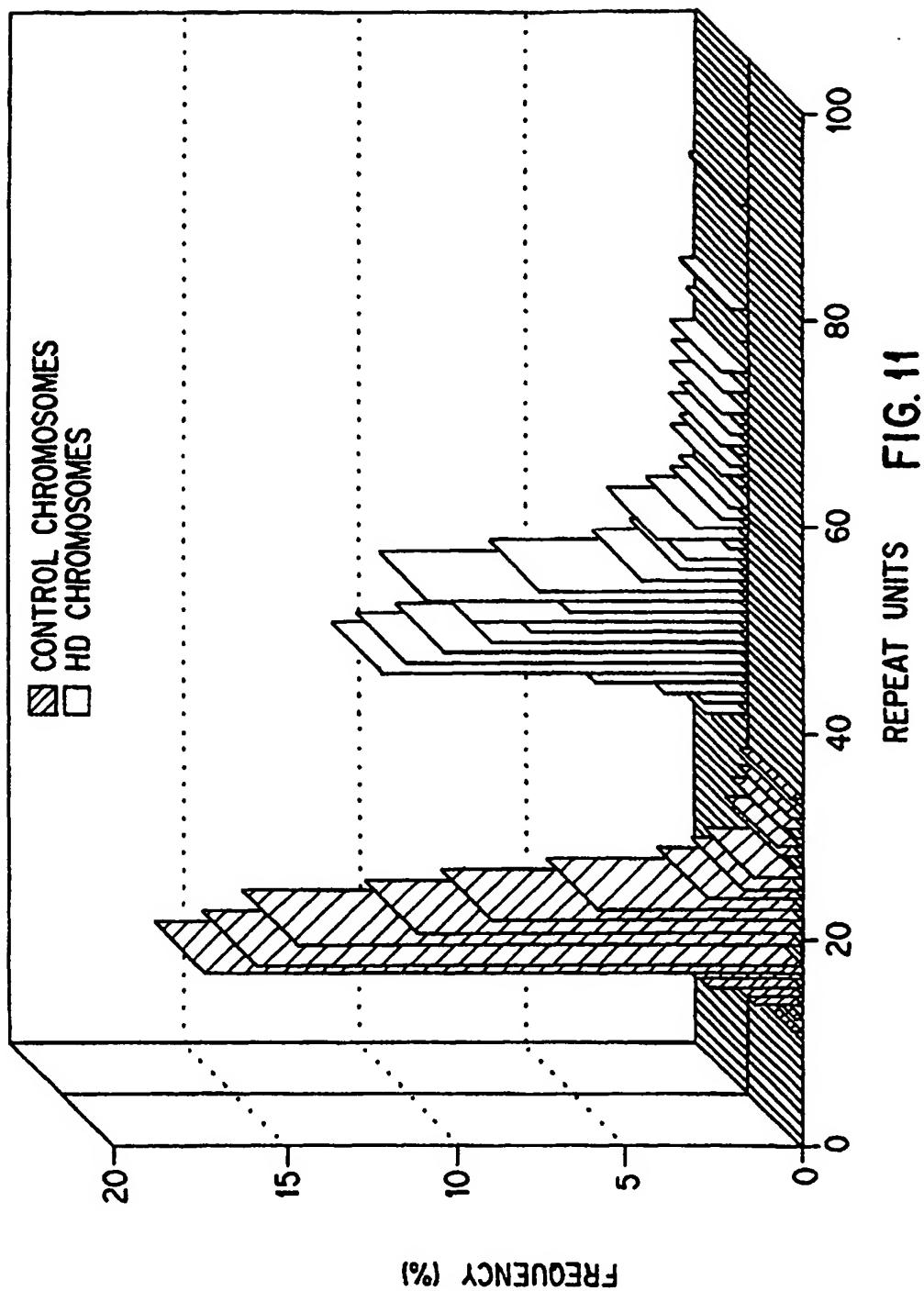


FIG. 11

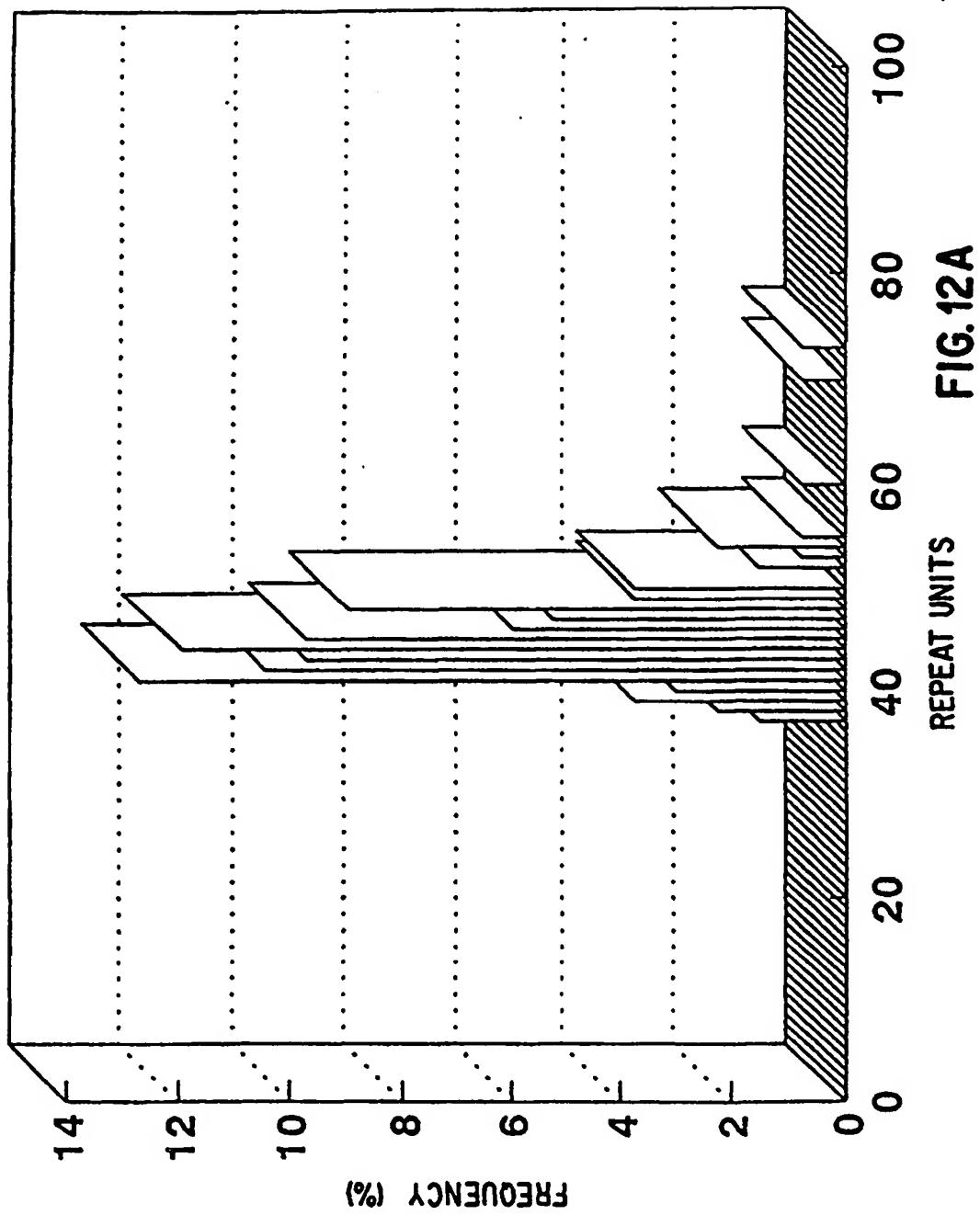
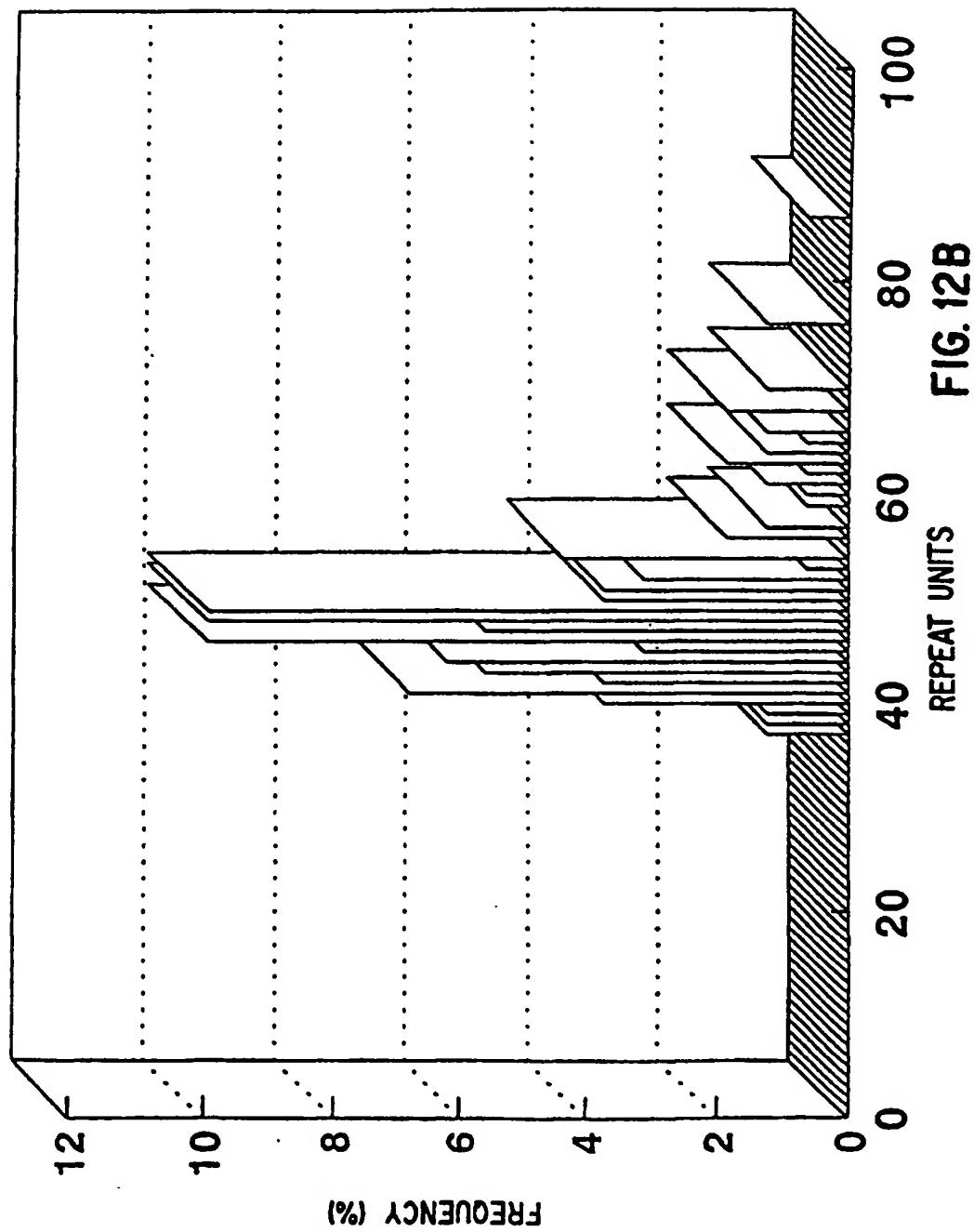
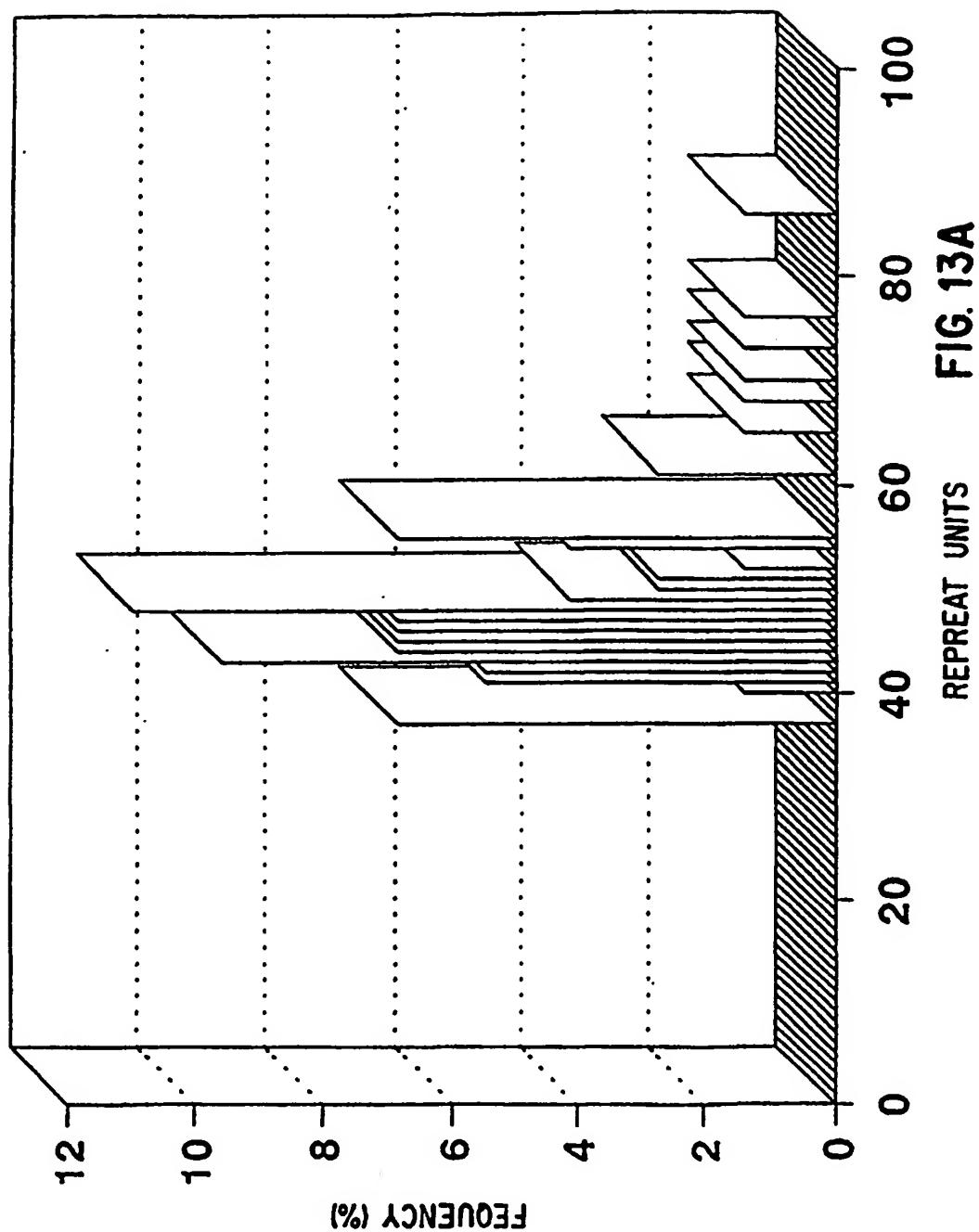


FIG. 12A





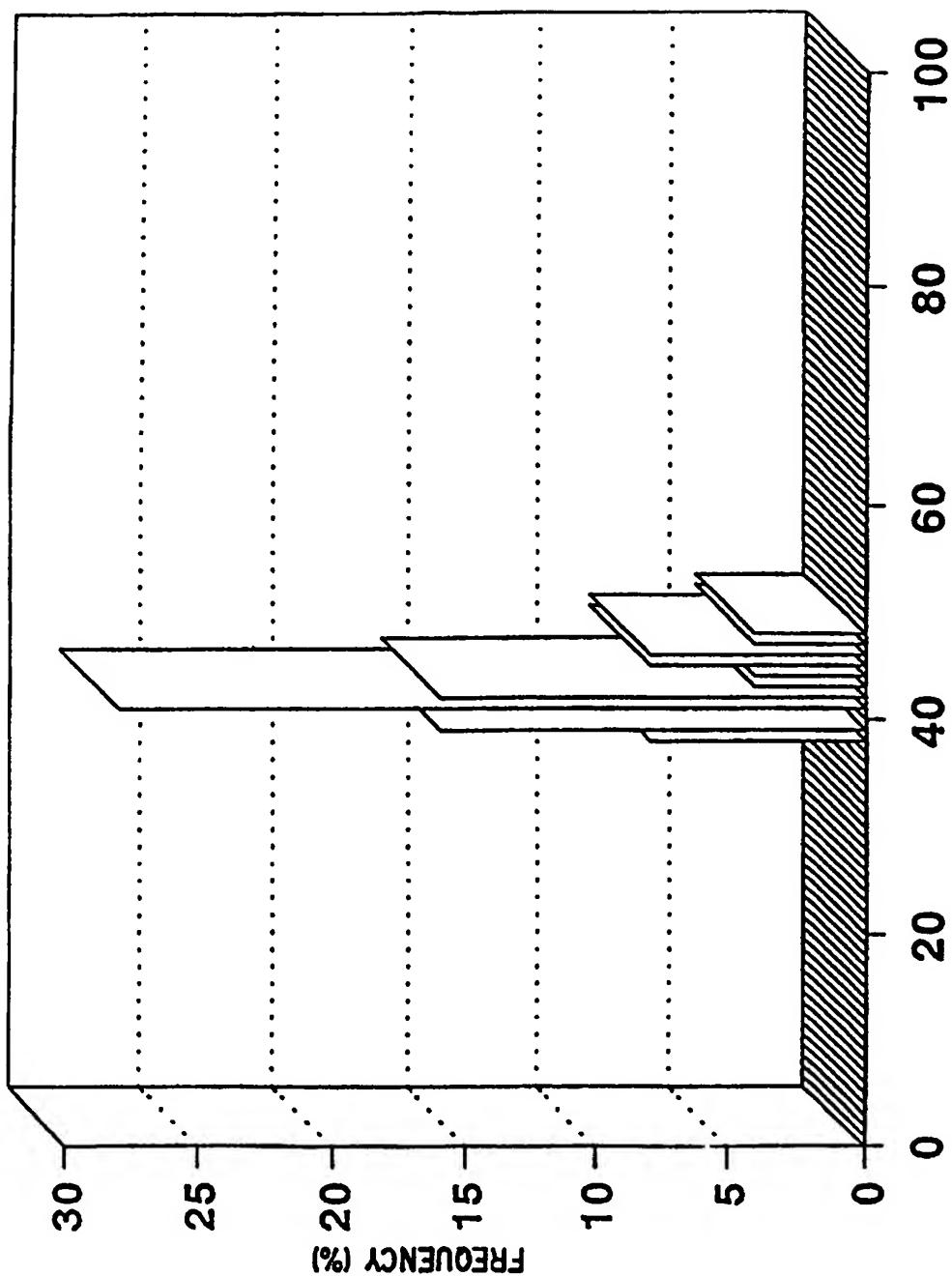
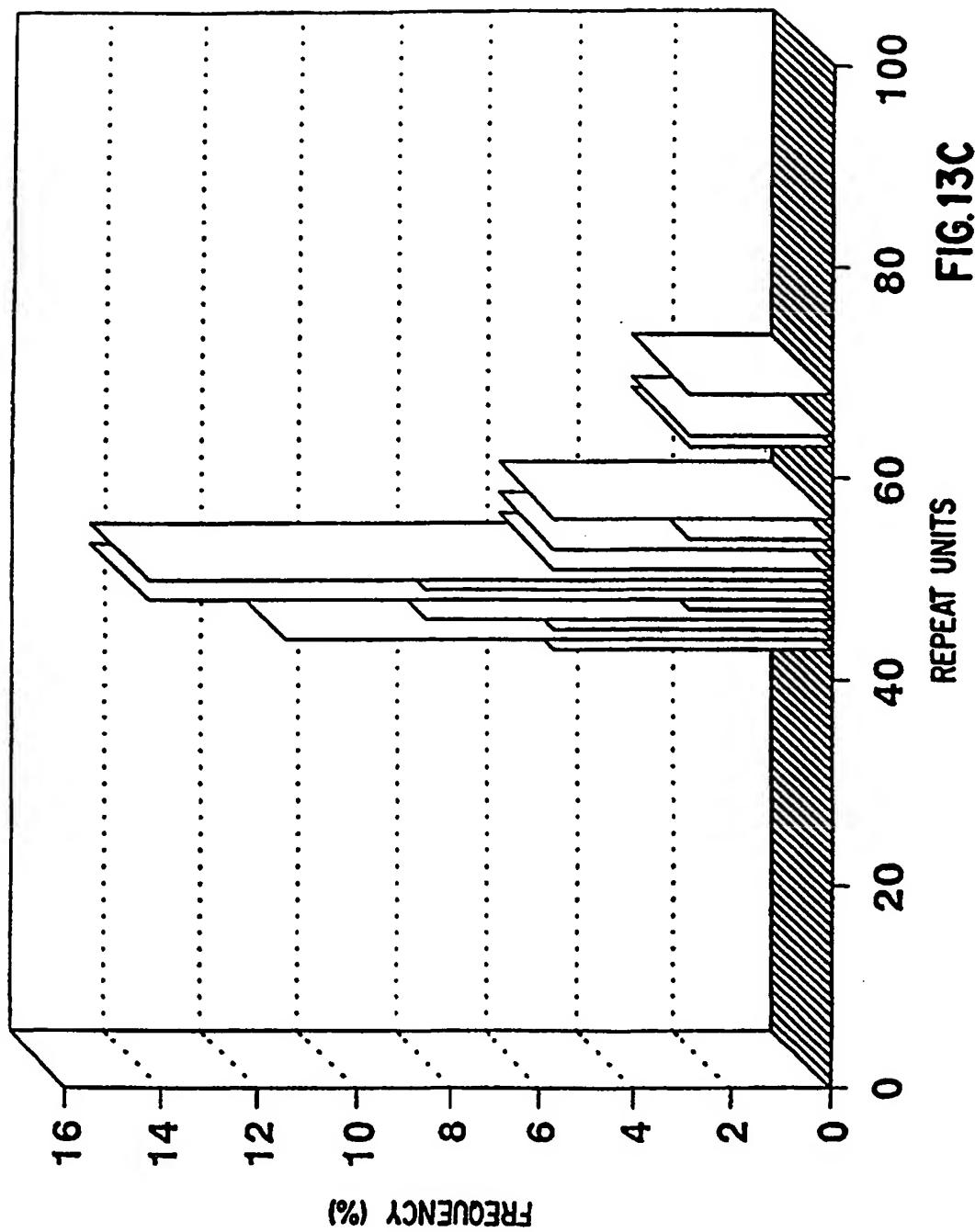


FIG. 13B



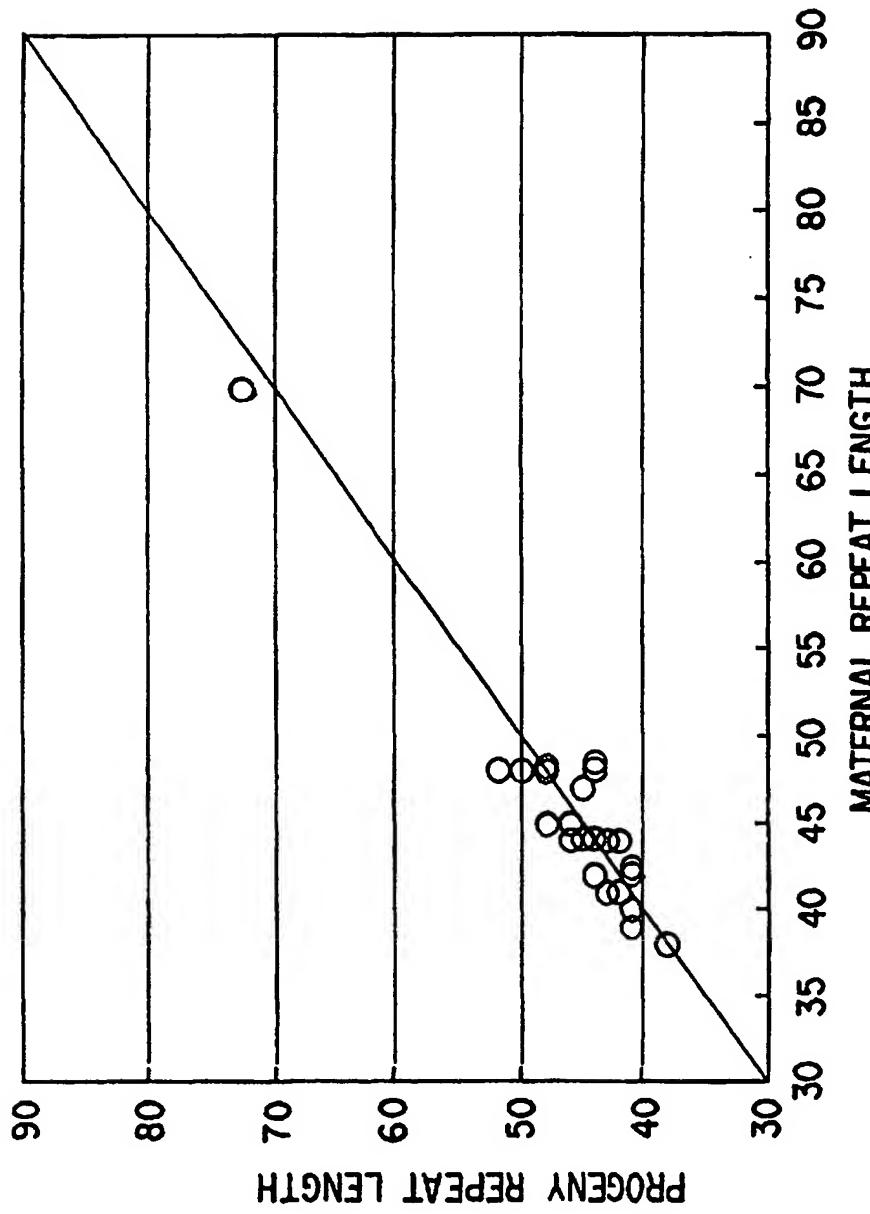
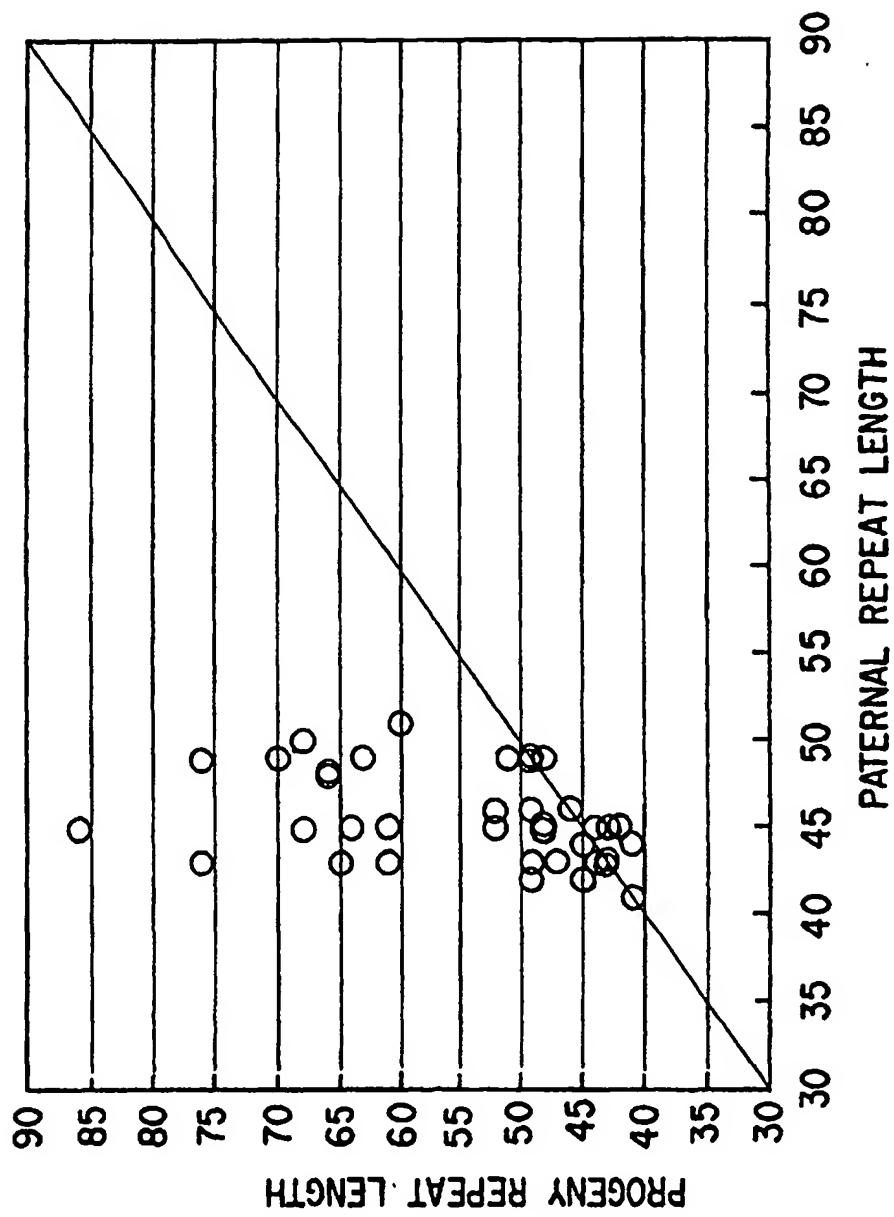
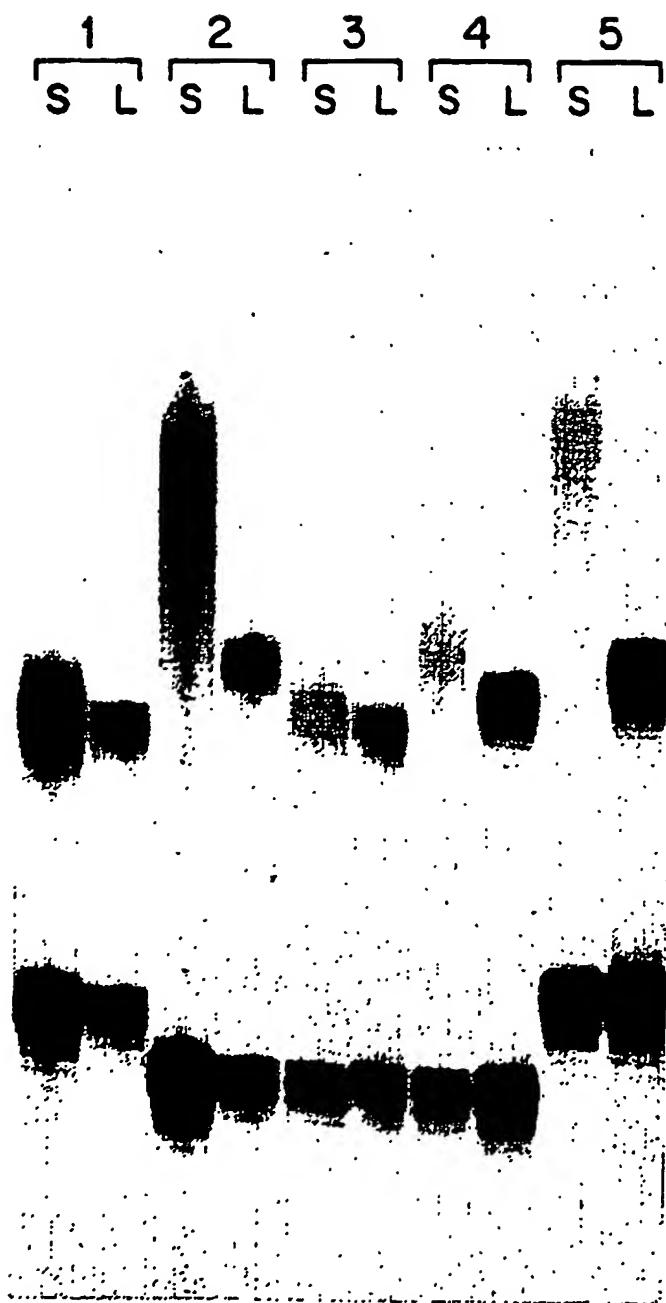


FIG. 14A





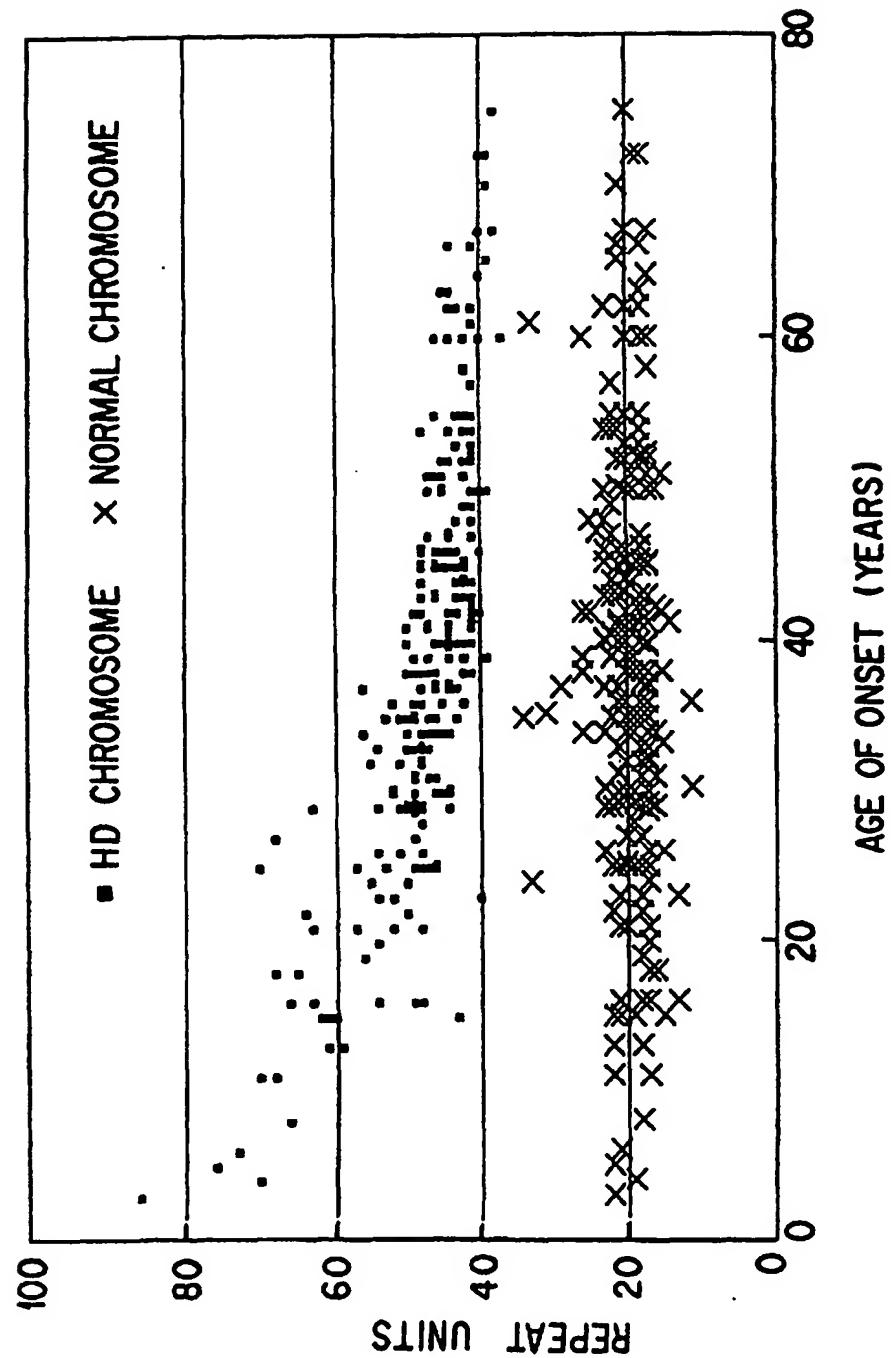


FIG. 16